

ggt (1-12-01)

ANNUAL REPORT

Division of Intramural Research Programs
National Institute of Mental Health (U.S.)

October 1, 1982 - September 30, 1983

VOLUME II INDIVIDUAL PROJECT REPORTS

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration
National Institute Of Mental Health
Division of Intramural Research Programs

KA

790.6

U5591

1983

v. 2

ANNUAL REPORT

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1982 - September 30, 1983

VOLUME II

INDIVIDUAL PROJECT REPORTS

ANNUAL REPORT
DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

October 1, 1982 - September 30, 1983

TABLE OF CONTENTS

VOLUME II - INDIVIDUAL PROJECT REPORTS

BIOLOGICAL PSYCHIATRY BRANCH

Office of the Chief

Z01 MH 00092-09 BP	Central Amines and Aggression, Suicide and Alcoholism.....	1
Z01 MH 00100-08 BP	Biobehavioral Aspects in Childhood and Adolescent Mental Illness.....	9

Section on Clinical Psychophysiology

Z01 MH 00034-13 BP	Psychological and Physiological Correlates of Average Evoked Response.....	17
Z01 MH 00035-11 BP	Biochemical and Psychopharmacological Correlates of the Averaged Evoked Response.....	19
Z01 MH 00036-09 BP	Individual Differences in Sleep and AER.....	21
Z01 MH 00039-09 BP	Sensory Thresholds and Averaged Evoked Responses.....	23
Z01 MH 00041-03 BP	Simultaneous Electroencephalography and Cerebral Glucography with PET in Normals and Schizophrenics.....	25

Section on Psychobiology

Z01 MH 00070-10 BP	Psychological and Biological Interactions in the Mood and Anxiety Disorders.....	27
Z01 MH 00071-03 BP	Psychobiological Correlates and Treatment of Panic and Related Mood Disorders.....	55
Z01 MH 00072-03 BP	Psychophysiological Investigation of Multiple Personality Disorder.....	69

Unit on Neuroendocrinology

Z01 MH 00180-01 BP	Psychobiology and Treatment of Menstrually-Related Mood Disorders.....	73
Z01 MH 00452-08 BP	Neuroendocrine Studies of Major Psychiatric Disorders.....	79

Unit on Behavioral Pharmacology

Z01 MH 00124-06 BP	Mechanism of Action of Lithium in the Treatment of Affective Disorders.....	95
Z01 MH 00147-08 BP	Behavioral and Physiological Effects of Brain Peptides and Other Psychoactive Compounds....	97

Section on Psychogenetics

Z01 MH 00081-09 BP	Heritable Characteristics of Cation Transport in Primary Affective Disorders.....	105
Z01 MH 00084-09 BP	Genetic-Biologic Studies of Psychiatric Disorders.....	109
Z01 MH 00085-07 BP	Pharmacogenetics of Psychoactive Drugs.....	119
Z01 MH 00086-07 BP	Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders.....	123
Z01 MH 00132-09 BP	Biological and Psychopharmacological Evaluation of Schizophrenia.....	129

CLINICAL NEUROPHARMACOLOGY BRANCH

Z01 MH 00326-10 CN	Clinical Neuropharmacology and Psychobiology of Depression and Mania.....	131
Z01 MH 00329-08 CN	Platelets and Other Systems as Models for the Study of Neurotransmitter Function.....	143
Z01 MH 00330-05 CN	Use of Electron and Photon Imaging Techniques to Study Aminergic Systems.....	151
Z01 MH 00331-05 CN	Use of Nuclear Magnetic Resonance to Study Aminergic Systems.....	157
Z01 MH 00332-05 CN	Animal Models for the Study of Neuropharmacologic Effects.....	163
Z01 MH 00335-05 CN	Smooth Pursuit Eye Tracking Impairment and its Relation to Psychopathology and CNS Disorders.....	169

Z01 MH 00336-04 CN	The Phenomenology and Treatment of Obsessive-Compulsive Disorder in Adults.....	171
Z01 MH 00337-04 CN	Neuropharmacology of Neuroendocrine and Neurotransmitter Regulatory Mechanisms.....	177
Z01 MH 00338-03 CN	Families of Origin in Obsessive-Compulsive Illness.....	183
Z01 MH 00339-02 CN	The Neuropharmacology of Cognition.....	187

CLINICAL PSYCHOBIOLOGY BRANCH

Inpatient Research Unit

Z01 MH 00446-14 CP	Inpatient Clinical Studies of Affective Illness.....	193
Z01 MH 00447-14 CP	Amine Neurotransmitters and Metabolites in Mental Illness.....	199
Z01 MH 00450-09 CP	Biological Rhythms in Affective Illness.....	205
Z01 MH 02196-01 CP	Causes of the Delayed Sleep Phase Syndrome.....	209
Z01 MH 02197-01 CP	Treatment of Delayed Sleep Phase Syndrome with Light.....	213
Z01 MH 02198-01 CP	Effect of Light on Free-Running Human Circadian Rhythms.....	217
Z01 MH 02199-01 CP	Circadian Rhythms in Affective Disorder Patients Isolated from Time Cues.....	221
Z01 MH 02200-01 CP	Light Suppression of Nocturnal Human Melatonin Secretion.....	225
Z01 MH 02201-01 CP	Early Versus Late Partial Sleep Deprivation in the Treatment of Depression.....	229

Outpatient Research Unit

Z01 MH 02202-01 CP	Clinical Features of Seasonal Affective Disorder (SAD).....	233
Z01 MH 02203-01 CP	Sleep, Temperature and Activity Changes in Women with Premenstrual Syndrome.....	237
Z01 MH 02205-01 CP	Effects of Light Interventions in Seasonal Affective Disorder (SAD).....	241

Z01 MH 02206-01 CP	Neurobiology of Seasonal Affective Disorder (SAD).....	245
--------------------	--------------------------------------------------------	-----

Unit on Clinical Pharmacology

Z01 MH 01850-06 CP	Clinical Pharmacology of Antidepressants....	249
--------------------	----------------------------------------------	-----

Unit on Family Studies

Z01 MH 00449-09 CP	Outpatient Followup Studies of Manic-Depressive Patients and Families.....	257
--------------------	----------------------------------------------------------------------------	-----

Unit on Sleep Studies

Z01 MH 02192-01 CP	Sleep in Psychiatric and Endocrine Disorders.....	263
Z01 MH 02193-01 CP	Clinical Studies of Insomnia.....	269
Z01 MH 02194-01 CP	Anticonvulsant/Proconvulsant Effects of Benzodiazepine Receptor Ligands.....	273
Z01 MH 02195-01 CP	Studies of the Physiology and Pharmacology of Sleep.....	277
Z01 MH 02204-01 CP	Studies of Sleep as a Circadian Rhythm.....	281

LABORATORY OF CLINICAL SCIENCE

Section on Pharmacology

Z01 MH 00422-12 LCS	Neuropharmacology of Circadian Rhythms.....	285
Z01 MH 00425-07 LCS	Peripheral and Central Catecholamines in Hypertension.....	287
Z01 MH 00427-06 LCS	Phospholipid Methylation and Signal Transduction.....	291
Z01 MH 00428-04 LCS	Protein Carboxyl-Methylation: A Post Translational Modifier of Protein Function.....	293
Z01 MH 00429-04 LCS	Biosynthesis of Nonpolar Methylated Lipids.....	295
Z01 MH 00433-03 LCS	Role of Neuropeptides in Neuroendocrine Regulation.....	297
Z01 MH 00434-02 LCS	Cellular Mechanisms of ACTH Secretion from Mouse Pituitary.....	301

Section on Histopharmacology

Z01 MH 00382-09 LCS	Localization and Characterization of Brain Neuropeptides.....	305
Z01 MH 00388-07 LCS	Colocalization of Substance P and Acetylcholinesterase in Neurons of the Brain.....	309
Z01 MH 00396-05 LCS	A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis.....	313
Z01 MH 00397-05 LCS	Neurophysiological Effects of Brain Peptides.....	317
Z01 MH 00400-01 LCS	Protein Phosphorylation in Brain.....	321
Z01 MH 01831-07 LCS	Basic and Clinical Studies of Neuronal and Glial Enolase.....	325
Z01 MH 01833-03 LCS	Adenosine Receptors in the Central Nervous Systems.....	331
Z01 MH 01834-06 LCS	Endogenous Ligands for the Brain Benzodiazepine Receptor.....	337

Section on Medicine

Z01 MH 00401-18 LCS	Peripheral Noradrenergic Function.....	339
Z01 MH 00402-11 LCS	CNS Regulation of Autonomic and Endocrine Function.....	345
Z01 MH 00403-10 LCS	Biochemical Indices of Adrenergic Function in Humans.....	349
Z01 MH 00404-12 LCS	Immunological Localization of GAD and CSD in Neurones and Other Cells.....	353
Z01 MH 00405-04 LCS	Regulation of Physiological Activity of Aminergic Receptors.....	355

Section on Analytical Biochemistry

Z01 MH 00274-09 LCS	Methods of Ionization in Mass Spectrometry.....	359
Z01 MH 00275-06 LCS	Release and Turnover of Catecholamine Metabolites in Human Subjects.....	363
Z01 MH 00276-04 LCS	Metabolism of Melatonin.....	365
Z01 MH 00277-04 LCS	Synthesis of Stable Isotope Labeled Compounds.....	369

Z01 MH 00279-01 LCS	Pharmacology of Neurotoxins.....	371
Z01 MH 00280-01 LCS	Studies in Morphine Tolerance.....	375

Section on Experimental Therapeutics

Z01 MH 00351-09 LCS	Clinical Pharmacology of the Central Nervous System.....	377
Z01 MH 00352-08 LCS	Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes.....	381
Z01 MH 00353-01 LCS	Biochemical and Pharmacological Studies of Parkinsonism and Related Disorders.....	389

Section on Child Psychiatry

Z01 MH 00153-06 LCS	The Treatment of Obsessional Children and Adolescents with Chlorimipramine.....	393
Z01 MH 00161-05 LCS	Behavioral Effects of Dietary Substances in Normal and Hyperactive Children.....	397
Z01 MH 00162-04 LCS	Treatment of Hyperactive Children with Desmethylinipramine.....	401
Z01 MH 00163-04 LCS	Naturalistic Study of Activity levels of Hyperactive Children.....	403
Z01 MH 00165-03 LCS	Biological Markers of Alcoholism.....	407
Z01 MH 00177-02 LCS	Treatment of Hyperactive Children with Monoamine Oxidase Inhibitors.....	411
Z01 MH 00178-02 LCS	Brain Structure and Function in Developmental Neuropsychiatric Disorders.....	415
Z01 MH 00301-01 LCS	Diagnosis in Child Psychiatry.....	419

Office of the Chief

Z01 MH 00271-14 LCS	Fate of 3-Methoxy-4-Hydroxy-Phenyl Glycol in Primates.....	423
---------------------	------------------------------------------------------------	-----

LABORATORY OF DEVELOPMENTAL PSYCHOLOGY

Z01 MH 00247-08 LDP	Patterns of Psychological Functioning in Children with Endocrine Abnormalities.....	427
Z01 MH 00257-07 LDP	Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia.....	429

Z01 MH 02135-06 LDP	Emotional Development in Children of Bipolar Depressed and Normal Parents.....	433
Z01 MH 02142-05 LDP	Behavioral Studies of Children with Juvenile Diabetes.....	437
Z01 MH 02144-03 LDP	Studies of Normal Families and Families with Pathology: A Research Paradigm.....	439
Z01 MH 02146-04 LDP	The Etiology of Problem Aggression in Early Childhood.....	443
Z01 MH 02147-04 LDP	Effects and Determinants of Parental Methods for Controlling Children's Behavior.....	447
Z01 MH 02148-04 LDP	Physiologic Jaundice as a Predictor of Behavioral Function in Preterm Infants.....	451
Z01 MH 02150-04 LDP	Adjustment to Stress in Early Adolescence: Environmental and Organismic Factors.....	455
Z01 MH 02152-04 LDP	Discipline and Parental Control in Families with Affective Disorders.....	459
Z01 MH 02153-04 LDP	Maternal Recall of Child's Early Experience.....	463
Z01 MH 02154-04 LDP	Stability and Change in Behavior Problems of Clinically Referred Children.....	467
Z01 MH 02155-04 LDP	Children of Depressed and Normal Parents....	471
Z01 MH 02156-04 LDP	Emotional-Social Development of Children Reared by Normal and Depressed Mothers.....	475
Z01 MH 02157-04 LDP	Developmental Evaluation of Infants on Chloride Deficient Diet.....	479
Z01 MH 02158-04 LDP	Impact of the Environment on the Development of the Abused Child.....	483
Z01 MH 02159-03 LDP	Information Processing and Adaptation to Research Hospitalization.....	487
Z01 MH 02161-03 LDP	Developmental Changes in Imitative Learning.....	491
Z01 MH 02163-03 LDP	Psychobiological Correlates of Behavior Problems.....	495
Z01 MH 02164-03 LDP	The Impact of Biological Changes on Psychological Functioning During Adolescence.....	497

Z01 MH 02165-01 LDP	Adjustment to Stress in Early Adolescence: Family and Peer Influence.....	501
Z01 MH 02166-01 LDP	Developmental Patterns of Cognition and Interaction in Children at Risk.....	503
Z01 MH 02167-01 LDP	Interpersonal Inferential Abilities in Normal and Depressed Mother-Child Pairs.....	507
Z01 MH 02168-01 LDP	Socialization in Early Infancy: Patterns of Interaction in Two Cultures.....	509
Z01 MH 02169-01 LDP	Interactions Between Siblings with a Depressed Parent.....	513
Z01 MH 02170-01 LDP	Psychiatric Evaluation of Infants and Toddlers.....	517

LABORATORY OF NEUROPSYCHOLOGY

Z01 MH 00478-27 LN	Neural Mechanisms of Memory and Habit Formation.....	521
Z01 MH 02032-07 LN	Neural Coding of Visual Stimuli in the Awake Monkey.....	527
Z01 MH 02033-06 LN	Functional Mapping of Sensory Systems.....	535
Z01 MH 02034-04 LN	Terminated.....	543
Z01 MH 02035-03 LN	Anatomy of the Primate Visual System.....	545
Z01 MH 02036-03 LN	Neural Coding of Visual Stimuli in the Immoblized Monkey.....	551
Z01 MH 02037-02 LN	Functional Anatomy of the Somatosensory Cortex of the Monkey.....	557
Z01 MH 02038-01 LN	Ontogenetic Development of Memory and Habit Formation.....	567
Z01 MH 02039-01 LN	Cholinergic Mechanisms in Memory.....	571

LABORATORY OF PSYCHOLOGY AND PSYCHOPATHOLOGY

Z01 MH 00471-28 LPP	Studies of Heredity and Environment in Schizophrenia.....	575
Z01 MH 00472-20 LPP	The Investigations of Some Formal Character- istics of Speech.....	581

Z01 MH 00484-23 LPP	Psychophysiological Responsivity and Behavior in Schizophrenia.....	583
Z01 MH 00486-11 LPP	Psychophysiological Concomitants of Minimal Brain Dysfunction in Children.....	591
Z01 MH 00491-07 LPP	Personality Factors and Psycho-Physiological Responses to Changing Stimulus Input.....	593
Z01 MH 00495-07 LPP	The Psychobiology of Cognitive Processes....	597
Z01 MH 00500-04 LPP	Cognitive and Perceptual Changes in Affective Illness.....	613
Z01 MH 00502-04 LPP	Atypicality in Major Depressive Illness....	619
Z01 MH 00503-03 LPP	Human Clinical Studies of Attention Disorders.....	621
Z01 MH 00504-03 LPP	Models in the Monkey of Generalized Seizures of the <u>Absence</u> Type.....	625
Z01 MH 00505-03 LPP	Brain Lesion and State Change Effects on Visual Attention.....	629
Z01 MH 00506-03 LPP	Attention-Related Neurons in the Brain of the Rhesus Monkey.....	635
Z01 MH 00507-01 LPP	Clinical Brain Imaging.....	639
Z01 MH 00508-01 LPP	Neuropsychological Evaluation of Psychiatric and Neurological Patients.....	645
Z01 MH 00509-01 LPP	Attention Disorders as Assessed by Event-Related Brain Potentials.....	649

LABORATORY OF SOCIO-ENVIRONMENTAL STUDIES

Z01 MH 00672-18 LSES	Social Psychological Correlates of Occupational Position.....	653
Z01 MH 00679-03 LSES	Structural Equation Models in the Analysis of Data with Measurement Error.....	675
Z01 MH 00680-01 LSES	Work Experiences and the Deinstitutionalized Mentally Ill.....	679

LABORATORY OF CELL BIOLOGY

Office of the Chief

Z01 MH 00424-08 LCB	Biologically Active Peptides in the Brain...	681
---------------------	----------------------------------------------	-----

Unit on Biochemical Pharmacology

Z01 MH 00422-12 LCB	Neuropharmacology of Circadian Rhythms.....	687
Z01 MH 00429-04 LCB	Biochemistry of Membranes.....	691

Unit on Biochemistry

Z01 MH 00427-06 LCB	On the Mechanism of Signal Transduction Through Receptors.....	695
---------------------	-------------------------------------------------------------------	-----

CLINICAL NEUROSCIENCE BRANCH

Section on Preclinical Studies

Z01 MH 01836-05 NS	Benzodiazepine Receptors in the Central Nervous System: Biochemistry to Behavior...	701
Z01 MH 02186-01 NS	Brain Recognition Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity.....	709

Section on Clinical Studies

Z01 MH 00112-06 NS	Endorphin Research in Mental Illness.....	715
Z01 MH 02181-01 NS	Neurobiology of Schizophrenia.....	721
Z01 MH 02184-01 NS	Biological Tests in Depression.....	727

Section on Brain Biochemistry

Z01 MH 00166-04 NS	Peptide Secretory Products of Oat Cell Carcinoma and Other Unicellular "Creatures".....	731
Z01 MH 00167-04 NS	Neurochemical Coding of Brain Pathways Revealed by Autoradiography.....	733
Z01 MH 00169-03 NS	Allosteric Receptor Modulation and Altered Sensitivity States.....	737
Z01 MH 02182-01 NS	Toward the Visualization of Opiate Receptors in Living Humans.....	739
Z01 MH 02183-01 NS	Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease.....	741
Z01 MH 02185-01 NS	What is the Etiology of Small Cell Carcinoma of Lung.....	745

Section on Molecular Pharmacology

Z01 MH 00177-08 NS	α -Adrenergic and Prostaglandin Receptors in Human Blood Elements.....	749
Z01 MH 00159-04 NS	Neurotransmitter Receptors in the Nervous System.....	753
Z01 MH 00179-02 NS	Morphological and Functional Aspects of Peptides in Mammalian Brain.....	757
Z01 MH 02177-01 NS	Behavioral Functions of Neuropeptides.....	763
Z01 MH 02178-01 NS	Neuropharmacology of Anxiety.....	767
Z01 MH 02179-01 NS	Hamster Separation Model of Depression.....	771
Z01 MH 02180-01 NS	Electrophysiological Studies of Peptidergic Function in Mammalian Brain.....	775

LABORATORY OF BRAIN EVOLUTION AND BEHAVIOR

Z01 MH 00851-19 LBEB	Brain Mechanisms of Display Behavior in Squirrel Monkey (<i>Saimiri sciureus</i>).....	779
Z01 MH 00787-04 LBEB	Brain Mechanisms of Isolation Call in Squirrel Monkey (<i>Saimiri sciureus</i>).....	783
Z01 MH 00793-02 LBEB	Brain Iron and Neuroendocrine Regulation....	789
Z01 MH 00871-07 LBEB	A Histological Study on the Location of Brain Iron.....	793
Z01 MH 00847-03 LBEB	Role of the Neocortex in Coping with Complexity.....	797
Z01 MH 00849-01 LBEB	Resting Time Residence in a 7-Generation Population of House Mice.....	803
Z01 MH 00850-01 LBEB	Cooperation Induced Modification of Behavior in Rats.....	809
Z01 MH 00848-02 LBEB	The Influence of Environmental Setting on Behavior and Population Dynamics.....	813

LABORATORY OF CEREBRAL METABOLISM

Section on Developmental Neurochemistry

Z01 MH 00881-27 LCM	Intermediary Energy Metabolism in Mammalian Brain.....	815
---------------------	-----------------------------------------------------------	-----

Z01 MH 00882-16 LCM	Studies on Regional Cerebral Circulation and Metabolism.....	827
Z01 MH 00887-06 LCM	The Extended Visual System of the Macaque Monkey.....	839
Z01 MH 00889-04 LCM	A Method for the Determination of Local Rates of Protein Synthesis in Brain.....	843

Section on Myelin Chemistry

Z01 MH 00900-27 LCM	Biochemical Studies on Myelin and Myelin Basic Protein.....	851
Z01 MH 00901-28 LCM	Immunologic Reactivity of Myelin Basic Protein.....	855
Z01 MH 00902-18 LCM	Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE).....	859
Z01 MH 00903-06 LCM	Improved Preparation of Human Myelin Basic Protein.....	865

LABORATORY OF GENERAL AND COMPARATIVE BIOCHEMISTRY

Z01 MH 00931-10 LGCB	Characteristics and Regulation of S-Adenosylhomocysteine Hydrolase.....	869
Z01 MH 00934-11 LGCB	The Biochemical Basis of Narcotic Drug Action.....	875
Z01 MH 00935-16 LGCB	Effect of Viruses and Plasmids on the Bio-Chemistry of Living Organisms.....	881
Z01 MH 00936-20 LGCB	Homocystinuria: Methionine Metabolism in Mammals.....	889
Z01 MH 00939-03 LGCB	Enzymes of Methionine Biosynthesis in Higher Plants.....	893
Z01 MH 00940-03 LGCB	Methionine Biosynthesis in Higher Plants....	897
Z01 MH 00941-03 LGCB	Biochemical Genetics and Metabolic Disease..	903
Z01 MH 00942-02 LGCB	Biochemical Reactions in Mammalian Cell Chemotaxis.....	911
Z01 MH 00943-02 LGCB	Pathways of Methionine and Threonine Metabolism and their Control in Higher Plants.....	915

LABORATORY OF NEUROBIOLOGY

Z01 MH 00981-18 LNB	Detection and Interpretation of Mechanical Changes in the Nervous System.....	919
Z01 MH 00983-05 LNB	Biochemical Studies on the Mechanism of Nerve Excitation.....	923
Z01 MH 01036-11 LNB	Regulation of Protein and Nerve Function by Modulator-Sites on Complex Carbohydrate....	927

LABORATORY OF NEUROCHEMISTRY

Z01 MH 01031-15 LNC	The Conversion of Phenylalanine to Tyrosine.....	933
Z01 MH 01034-15 LNC	The Biochemical Basis of Skeletal Muscle Hypertrophy.....	932
Z01 MH 01035-15 LNC	The Process of Lysogeny.....	941
Z01 MH 01037-14 LNC	The Role of the Cell Membrane in Cellular Organization, A Molecular Study.....	945
Z01 MH 01038-15 LNC	Phenylketonuria and Other Diseases Caused by Defects in Biopterin-Dependent Enzymes.....	949
Z01 MH 01039-15 LNC	Pteridine Biosynthesis.....	953

LABORATORY OF NEUROPHYSIOLOGY

Z01 MH 01081-13 LNP	Cerebral Control of Voluntary Movement.....	957
Z01 MH 01090-07 LNP	Studies of Central Nervous System Functional Anatomy.....	963
Z01 MH 01091-06 LNP	Motor Function in Patients with Neuro-psychiatric Disorders.....	969
Z01 MH 01092-05 LNP	The Non-Primary Motor Cortex and the Cerebral Control of Movement.....	975
Z01 MH 01093-05 LNP	Role of Somatic Sensory Inputs in the Cerebral Control of Movements.....	973
Z01 MH 01094-03 LNP	Information Processing in the Motor Cortex..	985

ADULT PSYCHIATRY BRANCH

Z01 MH 01335-13 SMRA	Studies of Schizophrenia.....	989
----------------------	-------------------------------	-----

Z01 MH 01337-12 SMRA	Studies on Drugs of Abuse.....	1025
Z01 MH 01338-05 SMRA	Studies of Aging.....	1033

LABORATORY OF PRECLINICAL PHARMACOLOGY

Z01 MH 01500-11 SMRP	Indolealkylamines and Neuronal Function.....	1047
Z01 MH 01503-09 SMRP	Pharmacological Studies of Acetylcholine Turnover: Control of Cholinergic Pathways..	1049
Z01 MH 01505-10 SMRP	Neurotransmitter Dynamics: Chlordecone.....	1051
Z01 MH 01506-09 SMRP	Narcotic Analgesics and the Regulation of Catecholamine Neurons.....	1053
Z01 MH 01508-13 SMRP	Modulation of Brain Cholinergic Function by Neuromodulators and Neuroactive Compounds...	1057
Z01 MH 01509-13 SMRP	Psychopharmacological Studies of Acetylcholine Turnover: Behavior.....	1059
Z01 MH 01510-08 SMRP	Effect of Cannabinoids on Cholinergic and GABAergic Dynamics in Rat Brain.....	1063
Z01 MH 01512-10 SMRP	Transmitter Interactions in the Regulation of Pituitary Function.....	1065
Z01 MH 01514-11 SMRP	Trans-Synaptic Control of Protein Synthesis.	1067
Z01 MH 01515-10 SMRP	Biochemical Changes in Cerebellum After Treatment with Psychoactive Drugs.....	1071
Z01 MH 01516-10 SMRP	Biochemical Pharmacology of Minor Tranquilizers.....	1073
Z01 MH 01518-07 SMRP	The Study of Mammalian GABAergic and Glutamatergic Mechanisms.....	1077
Z01 MH 01521-08 SMRP	Functional Role of Substance P and Other Peptides in Nervous System.....	1079
Z01 MH 01524-08 SMRP	Evidence for Peripheral Dopaminergic Neurons.....	1081
Z01 MH 01525-07 SMRP	Regulation of Gene Expression and Protein Synthesis of Neural Tissues.....	1083
Z01 MH 01526-07 SMRP	Retina: A Model for Studying Synaptic Biochemistry.....	1087
Z01 MH 01527-07 SMRP	Studies of Endogenous Opioids Using HPLC....	1091

Z01 MH 01531-06 SMRP	Nerve Growth Factors: Synthesis and Function.....	1093
Z01 MH 01532-06 SMRP	Regulation of Catecholamine Receptor.....	1095
Z01 MH 01536-05 SMRP	Characterization of Receptors for Putative Neurotransmitters.....	1099
Z01 MH 01537-05 SMRP	Biochemical Pharmacology of GABA Receptor System.....	1103
Z01 MH 01549-04 SMRP	Regulation of Imipramine Binding Sites in Rat Brain.....	1107
Z01 MH 01550-03 SMRP	Biochemical Mechanisms Regulated by Various Receptors in Anterior Pituitary.....	1111
Z01 MH 01551-03 SMRP	Is Insulin a Neuromodulator in the Central Nervous System?.....	1115
Z01 MH 01552-03 SMRP	Agonist and Antagonist of Benzodiazepine Receptors.....	1117
Z01 MH 01553-03 SMRP	Biosynthesis of Enkephalins in Bovine Adrenal Medulla.....	1121
Z01 MH 01555-03 SMRP	Enkephalin Metabolism.....	1125
Z01 MH 01556-03 SMRP	Release of Endorphins.....	1129
Z01 MH 01558-02 SMRP	Immunohistochemical Studies on Neurotransmitters in the Nervous System.....	1131
Z01 MH 01559-02 SMRP	Met ⁵ -enkephalin-arg ⁶ -phe ⁷ in the Brain and Spinal Cord.....	1135
Z01 MH 01560-02 SMRP	Met ⁵ -enkephalin-arg ⁶ -phe ⁷ in Peripheral Tissue.....	1139
Z01 MH 01561-02 SMRP	Cholecystokinin in Brain.....	1141
Z01 MH 01562-02 SMRP	The Cholinergic Neuronal System.....	1143
Z01 MH 01563-02 SMRP	Adenosine: A Putative Neurotransmitter.....	1145
Z01 MH 01564-02 SMRP	Control of GABA Turnover in Rat Striatum....	1149
Z01 MH 01565-02 SMRP	Regulation of GABA _A and GABA _B Receptor Function.....	1151
Z01 MH 01566-02 SMRP	Molecular Mechanisms in the Antidepressant Action of (-)Deprenyl.....	1155

Z01 MH 01567-01 SMRP	Role of Synaptosomal Basic Proteins in the Control of GABA Receptor Function.....	1159
Z01 MH 01568-01 SMRP	Sulfacation of Cholecystokinin and Other Brain Peptides.....	1161
Z01 MH 01569-01 SMRP	Interaction with Neuropeptides.....	1163
Z01 MH 01570-01 SMRP	Synthesis of the Neurotransmitter Pool of Glutamate in the Brain.....	1165
Z01 MH 01571-01 SMRP	GABA/Benzodiazepine Receptor Complex in Adrenal Medulla.....	1167
Z01 MH 01572-01 SMRP	Endogenous Effector for Benzodiazepines.....	1169
Z01 MH 01573-01 SMRP	In Vivo Neurotransmitter Receptor Binding: Model for Emmission Computed Tomography.....	1173
Z01 MH 01574-01 SMRP	Peptide Ligands for Nicotinic Receptors.....	1177

DIVISION OF INTRAMURAL RESEARCH PROGRAMS

NATIONAL INSTITUTE OF MENTAL HEALTH

RESEARCH PROJECT SERIAL NUMBER LISTING:

Z01MH00034	Z01MH00335	Z01MH00509
Z01MH00035	Z01MH00336	Z01MH00672
Z01MH00036	Z01MH00337	Z01MH00679
Z01MH00039	Z01MH00338	Z01MH00680
Z01MH00041	Z01MH00339	Z01MH00787
Z01MH00070	Z01MH00351	Z01MH00793
Z01MH00071	Z01MH00352	Z01MH00847
Z01MH00072	Z01MH00353	Z01MH00848
Z01MH00081	Z01MH00382	Z01MH00849
Z01MH00084	Z01MH00388	Z01MH00850
Z01MH00085	Z01MH00396	Z01MH00851
Z01MH00086	Z01MH00397	Z01MH00871
Z01MH00092	Z01MH00400	Z01MH00881
Z01MH00100	Z01MH00401	Z01MH00882
Z01MH00112	Z01MH00402	Z01MH00887
Z01MH00117	Z01MH00403	Z01MH00889
Z01MH00124	Z01MH00404	Z01MH00900
Z01MH00132	Z01MH00405	Z01MH00901
Z01MH00147	Z01MH00422	Z01MH00902
Z01MH00153	Z01MH00424	Z01MH00903
Z01MH00159	Z01MH00425	Z01MH00931
Z01MH00161	Z01MH00427	Z01MH00934
Z01MH00162	Z01MH00428	Z01MH00935
Z01MH00163	Z01MH00429	Z01MH00936
Z01MH00165	Z01MH00433	Z01MH00939
Z01MH00166	Z01MH00434	Z01MH00940
Z01MH00167	Z01MH00446	Z01MH00941
Z01MH00169	Z01MH00447	Z01MH00942
Z01MH00177	Z01MH00449	Z01MH00943
Z01MH00178	Z01MH00450	Z01MH00981
Z01MH00179	Z01MH00452	Z01MH00983
Z01MH00180	Z01MH00471	Z01MH01031
Z01MH00247	Z01MH00472	Z01MH01034
Z01MH00257	Z01MH00478	Z01MH01035
Z01MH00271	Z01MH00484	Z01MH01036
Z01MH00274	Z01MH00486	Z01MH01038
Z01MH00275	Z01MH00491	Z01MH01039
Z01MH00276	Z01MH00495	Z01MH01081
Z01MH00277	Z01MH00500	Z01MH01090
Z01MH00279	Z01MH00502	Z01MH01091
Z01MH00280	Z01MH00503	Z01MH01092
Z01MH00301	Z01MH00504	Z01MH01093
Z01MH00326	Z01MH00505	Z01MH01094
Z01MH00329	Z01MH00506	Z01MH01335
Z01MH00330	Z01MH00507	Z01MH01337
Z01MH00331	Z01MH00508	Z01MH01338
Z01MH00332		

RESEARCH PROJECT SERIAL NUMBER LISTING (Cont.)

Z01MH01500	Z01MH02034	Z01MH02206
Z01MH01503	Z01MH02035	
Z01MH01505	Z01MH02036	
Z01MH01506	Z01MH02037	
Z01MH01508	Z01MH02038	
Z01MH01509	Z01MH02039	
Z01MH01510	Z01MH02135	
Z01MH01512	Z01MH02142	
Z01MH01514	Z01MH02144	
Z01MH01515	Z01MH02146	
Z01MH01516	Z01MH02147	
Z01MH01521	Z01MH02148	
Z01MH01524	Z01MH02150	
Z01MH01525	Z01MH02152	
Z01MH01526	Z01MH02153	
Z01MH01527	Z01MH02154	
Z01MH01531	Z01MH02155	
Z01MH01532	Z01MH02156	
Z01MH01536	Z01MH02157	
Z01MH01537	Z01MH02158	
Z01MH01549	Z01MH02159	
Z01MH01550	Z01MH02161	
Z01MH01551	Z01MH02163	
Z01MH01552	Z01MH02164	
Z01MH01553	Z01MH02165	
Z01MH01555	Z01MH02166	
Z01MH01556	Z01MH02167	
Z01MH01558	Z01MH02168	
Z01MH01559	Z01MH02169	
Z01MH01560	Z01MH02170	
Z01MH01561	Z01MH02180	
Z01MH01562	Z01MH02181	
Z01MH01563	Z01MH02182	
Z01MH01564	Z01MH02183	
Z01MH01565	Z01MH02184	
Z01MH01566	Z01MH02185	
Z01MH01567	Z01MH02186	
Z01MH01568	Z01MH02192	
Z01MH01569	Z01MH02193	
Z01MH01570	Z01MH02194	
Z01MH01571	Z01MH02195	
Z01MH01572	Z01MH02196	
Z01MH01573	Z01MH02197	
Z01MH01574	Z01MH02198	
Z01MH01831	Z01MH02199	
Z01MH01833	Z01MH02200	
Z01MH01834	Z01MH02201	
Z01MH01836	Z01MH02202	
Z01MH01850	Z01MH02203	
Z01MH02032	Z01MH02204	
Z01MH02033	Z01MH02205	

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00092-09 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Central Amines and Aggression, Suicide, and Alcoholism		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Gerald L. Brown, M.D.,		
COOPERATING UNITS (if any) Laboratory of Clinical Studies, NIAAA; Department of Psychiatry National Naval Medical Center; Portsmouth Naval Medical Center; IRP, NIMH		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .90	PROFESSIONAL: .60	OTHER: .30
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The National Institute of Mental Health (NIMH), both separately and together with the National Naval Medical Center, is studying <u>central amine metabolites</u> in the <u>cerebrospinal fluid</u> (CSF) of psychiatric patients. Results to date indicate that aggression and anti-social behavior are inversely correlated with <u>CSF 5-hydroxyindoleacetic acid (5HIAA)</u> . Low CSF 5HIAA is also associated with <u>suicidal history</u> ; <u>suicidal history</u> is similarly associated with a history of aggressive, anti-social behavior. Findings have been largely replicated in two separate populations. Alcoholics have decreased CSF 5HIAA during abstinence. Disulfiram (Antabuse) appears to lower CSF homovanillic acid (HVA) and appears to increase serum norepinephrine (NE); low CSF dopamine-beta-hydroxylase (DBH), low platelet monoamine oxidase (MAO), low plasma amine oxidase (AO), and high red-cell catechol-O-methyl transferase (COMT) are related to adverse reactions to disulfiram. CSF DBH is inversely related to significant deviations in certain personality measures on the MMPI; CSF 5HIAA is inversely related to the Pd scale. A trivariant relationship exists between <u>history of aggression</u> , <u>history of suicidal behavior</u> , and lower CSF 5HIAA.		

Collaborators:

Dr. Frederick Goodwin, Scientific Director, IRP, NIMH

Lt. Cmdr. Peter F. Goyer, M.D., Dept. of Psychiatry, Portsmouth Naval Medical
Center

Dr. Markku Linnoila, Chief, Laboratory of Clinical Studies, NIAAA

Captain O.L. Royal, MC, USN, National Naval Medical Center

Project Description:

Objectives: Evidence obtained in recent years indicates that epinephrine, norepinephrine (NE), dopamine, serotonin (5HT), acetylcholine, and gamma-amino butyric acid (GABA), among others, act as neurotransmitters and/or neuromodulators of the central nervous system (CNS). Although considerable indirect pharmacologic evidence has linked these amine systems with psychiatric illness (particularly affective illness and schizophrenia), the relative lack of direct data in man has limited the assessment of linkages to improved diagnosis, though our work raises the possibility that searching for interrelationships between central biochemical functioning and repeated behavioral patterns may be more fruitful than searching for traditional diagnostic specificity of biochemical findings. In any case, further confirmation of relationships between central biochemistry and behavior could lead to more specific pharmacological treatments. Direct data from man can be immensely valuable in making use of the massive data from animals and assessing the differences and similarities between man and animals. There has been a relative dearth of data on central neurochemical function in the various personality disorders -- a rather striking deficit in our knowledge considering the evidence suggesting that some personality disorders, particularly those involving criminality, have patterns of a genetic component. Furthermore, certain patterns of behavior often seen within personality disorders, i.e., depression, alcoholism and suicide, also appear to have genetic components. Data from animals suggests a relationship between aggressive behavior and neurotransmitters. Neurochemical studies in human alcoholism have also been limited. A purpose of this project is to extend the studies of central amine turnover into larger and more diverse populations of psychiatric patients and to assess behavioral-biochemical relationships not limited to specific diagnostic categories. Dr. Fred Goodwin continues to provide current scientific supervision on this multi-faceted project.

Methods Employed: Independent studies are both a separate effort of NIMH and a joint effort with the National Naval Medical Center, both in Bethesda, MD. Two study groups consisted entirely of military, active duty, inpatient males of normal intelligence; the first study was comprised of 26 subjects and the second, 12. More patients were not available for the second study. The two groups were of the same age range (17 to 32 years) and of a similar mean age (mean \pm SD = 22.1 ± 3.6 and 22.0 ± 5.2 , respectively). Height was unavailable in the first study, but ranged from 68 to 73 inches in the second study (70.6 ± 1.4). All study subjects were unpaid volunteers from whom informed consent was obtained. Patients were excluded from the two studies if medical disorders were present or if there was evidence of past or current primary affective disorder or schizophrenia, or if other than transient organic brain syndrome had ever been observed. An important clinical difference between the two groups, however, was that any presence or history of psychotic symptomatology was a basis for exclusion from the first study group; whereas a history of Brief, Reactive Psychosis (DSM-III, No. 298.80) as secondary diagnosis was present in four of the second study group and two others had had episodes of severe withdrawal sufficient to meet the criteria for Schizoid Personality Disorder (DSM-III, No. 301.20) as a secondary diagnosis. Clinical diagnoses and clinical history assessments were made independently of biochemical investigations. Further exclusion criteria were the ingestion of any drug, prescribed or illicit, within ten days of a scheduled lumbar puncture (LP) and heavy use of

alcohol (a score of greater than 6 on the Michigan Alcoholism Screening Test [MAST]). Alcoholic study groups were somewhat older and did score greater than 6 on the MAST. They did not have significant histories of medical, affective, schizophrenic, or organic disorders. Material available for evaluating each patient included full psychiatric/medical history, physical examination, and job performance assessments. Since a purpose of admission was evaluation of suitability for further military service, emphasis was given to a life history of aggression, particularly in response to authority. The categories of behavior used to determine aggression history, its scoring, its reliability, and its use in a normal age- and sex-matched control group have been described in detail in published studies. In addition, the Buss-Durkee Inventory (BDI) for aggression and the Minnesota Multiphasic Personality Inventory (MMPI) have been used. Individual items of the psychopathic deviate (Pd) scale of the MMPI approximate behaviors reflected in the life history of aggression measure. The use of standardized personality assessment instruments should facilitate attempts at further replication. All evaluative and behavioral data were collected, scored, and analyzed independently of the biochemical data.

Cerebrospinal fluid (CSF) was obtained from study group subjects following the procedures developed and revised at NIH and elsewhere. Assay details are described in the published studies. Other studies in conjunction with pharmacological interventions have further provided knowledge of functional brain chemistry in relationship to behavior, diagnosis and personality.

Currently, new protocols within the Biological Psychiatry Branch and in collaboration with Dr. Robert M. Post are being developed to characterize more clearly those patients who are at risk for aggressive and suicidal behavior. In brief, subjects will have their indoleamine metabolism assessed directly and indirectly in several ways, i.e., repeated LP's, tritiated imipramine binding and 5HT in platelets, a fenfluramine challenge, and oral tryptophan pharmacokinetics. In addition, glucose tolerance testing and chromosomal assessment will be done.

Major Findings: Initial results from personality disorders with problems secondary to poor impulse control, high levels of anger-hostility, and poor judgment indicated that aggressive behavior is inversely correlated with 5-hydroxyindoleacetic acid (5HIAA) and positively correlated with 3-methoxy-4-hydroxyphenylglycol (MHPG). Personality disorders have shown no significant difference in CSF cyclic 3',5'-adenosine monophosphate (c-AMP) from neurological patients with non-CNS disorders or from depressive, manic, and schizophrenic patients. Aggressive behavior was positively correlated with c-AMP and c-GMP in one group but not in a second. Those who were administratively discharged from the Service and those with history of suicidal attempts had lower CSF 5HIAA and higher MHPG, c-AMP, and c-GMP. Borderline personalities (DSM-III) show an inverse relationship between CSF 5HIAA and the Pd scale, as well as a history of aggressive behavior; neither the MHPG relationships nor the cyclic nucleotide relationships were replicated. This study of c-AMP and c-GMP in borderlines has not yet been published. The trends are the same as those seen in the first study. Some of the differences between studies that may account for the non-replication of the MHPG and cyclic nucleotide findings are differences in diagnoses and homogeneity of intra-group behavioral patterns, smaller numbers of patients in the second study, and later refinements in assay methods. A

trivariant relationship between a history of aggression, history of suicidal behavior, and lower CSF 5HIAA is readily apparent.

In that aggressive behavior has been shown in animals to be associated with lower GABA, new studies of CSF GABA, both free and bound, are being analyzed in the borderline group of patients. Of further interest is the initiation of new protocols (above) and the assessment of patients who are accused of murder and have a history of impulsive behavior. As our experience accumulates, the aggressive variable that appears to be most likely associated with lower CSF 5HIAA is that characterized by lability of affect, history of repeated impulsivity, and explosiveness. Similarly, we intend to study individuals with histories of repeated suicidal behavior in themselves and their families; similarly, our experience and that of others appears to indicate that suicidality associated with aggressivity is most likely to be associated with reduced levels of CSF 5HIAA. Pilot trials of the new protocols have been completed but results are too preliminary to be reported at this time. Further collaboration with Dr. Goyer, USN, involves assessment of suicidality ³H-IMI, 5HT, and MAO in early teenagers, but no results are yet available.

Alcoholics do not differ from personality disorders in CSF HVA. However, mean CSF 5HIAA is higher in the intoxication-withdrawal stage and decreases over time in abstinence to reach a mean level not differing from that of personality disorders. Although CSF HVA levels do not change post-intoxication-withdrawal, these levels are depressed by disulfiram (Antabuse), a dopamine-beta-hydroxylase (DBH) inhibitor. Disulfiram use also correlates with an increase in serum NE. Mean serum DBH in alcoholics vs. normal controls was lower, blood pressure was higher, and serum NE was not different. Disulfiram is also associated with an increase in cholesterol in alcoholics. Lower CSF DBH is correlated with increasing psychopathology as measured by the MMPI and lower CSF DBH is associated with disulfiram-induced psychoses. Furthermore, low platelet monoamine oxidase (MAO), low amine oxidase (AO), and elevated erythrocyte catechol-O-methyltransferase (COMT) are associated with disulfiram-induced psychoses. New studies show that neither clinical depression nor aggressive behavior in this group of early to mid-stage alcoholics can be associated with alcoholism; nor can improvement in depression or anxiety ratings of hospitalized alcoholics be attributed to disulfiram.

The above represents both studies that have been earlier published, those in press, and those in preparation. Continued collaboration with Dr. Linnoila of NIAAA involves the new protocols on aggression and suicidality, as well as collaboration on alcoholics to be admitted to NIAAA. With regard to both groups, chromosomal studies and serum electrophoresis studies are underway, but results are preliminary.

Significance to Mental Health Research: CNS functioning is greatly understudied in some major groups of psychiatric patients, viz. personality disorders, alcoholics, and borderlines. Studies of animal models, as well as Gilles de la Tourette syndrome, hyperactive children, and prisoners suggest a relationship between central neurotransmitter systems and aggressive behavior. Human suicidal behavior has an enormous public health and social significance and, previously, had largely been studied from a psychological and sociological point of view. These studies lead to the possibility of identifying those at risk for

anti-social and suicidal behaviors and possibly altering these behaviors through neuropharmacological adjuncts to management of the psychiatric and/or behavioral problems. The neurobiological aspects of alcoholism, either predisposing, concomitant, or resultant, are of timely significance as alcoholism is a prevalent problem. Also, drug-free personality disorders may serve as a useful comparison group for biological studies of other psychiatric disorders.

Proposed Course of Project: The preparation for this project began in January 1973. The approval processes, both in terms of scientific merit and the protection of rights of patients, were completed in July 1974. The first lumbar puncture was performed in September 1974. The progress of the project has been submitted to the Navy for reapproval each March and has now been terminated with regard to obtaining new subjects. We believe this collaboration continues to be of mutual benefit to NIMH and NNMC. There is still some neurochemical, behavioral, and psychological data to be analyzed and reported from the patients who have participated in these studies. Additionally, new protocols of a similar nature are being prepared, as described above, to continue this work within the BPB.

Publications:

Brown, G.L., Goodwin, F.K., and Bunney, W.E., Jr.: Human aggression and suicide: their relationship to neuropsychiatric diagnoses and serotonin metabolism. In Ho, B.T., Usdin, E., and Bunney, W.E., Jr. (Eds.): Serotonin in Biological Psychiatry, Advances in Biochemical Psychopharmacology, Vol. 34. New York, Raven Press, 1982, pp. 287-307.

Brown, G.L., and Goodwin, F.K.: Aggression, adolescence and psychobiology. In Keith, C. (Ed.): Understanding and Treatment of the Aggressive Adolescent. New York, McMillan, 1983, in press.

Goyer, P.F., Brown, G.L., Minichiello, M.D., and Major, L.F.: Mood-altering effects of disulfiram (Antabuse) in alcoholics. J. Stud. Alcohol, 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00100-08 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biobehavioral Aspects in Childhood and Adolescent Mental Illness		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Gerald L. Brown, M.D. Medical Officer BPB NIMH		
COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS, NIMH; Communicative Disorders Program, NINCDS; Section on Child Psychiatry, LCS, NIMH		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.50	PROFESSIONAL: 1.00	OTHER: 3.50
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> An inpatient program with selected overnight stays, for <u>childhood and adolescent neuropsychiatric disorders</u> is ongoing. The condition currently under study is that of <u>hyperactive children (HAC)</u>. Pharmacological compounds under study in these disorders include <u>methylphenidate</u>, <u>amphetamine</u>, <u>piribedil</u>, <u>L-DOPA</u>, <u>tryptophan</u>, <u>Mianserin</u>, <u>clorgyline</u>, and <u>desipramine</u>. Piribedil is safe but clinically ineffective in HAC while L-DOPA is minimally clinically effective. Tryptophan is effective on attention measures. <u>Pharmacokinetic studies</u> with clinical responses are included. Amphetamine half-life in children is about one-third that of adults. Behavior and motor activity responses to d-amphetamine occur during the absorption phase as determined by serial plasma amphetamine following a single dose. <u>Central neurotransmitters</u> and their metabolites are being studied in plasma and urine. Urinary <u>3-methoxy-4-hydroxyphenylglycol (MHPG)</u> shows a time-related decrease during treatment with d-amphetamine; <u>dopamine</u> metabolites are unchanged. <u>Tyramine</u> and its metabolites are also decreased following d-amphetamine, whereas <u>phenylethylamine</u> is greatly increased following d-amphetamine. </p>		

Collaborators:

Dr. Michael H. Ebert, Chief, Section of Experimental Therapeutics, LCS and
Clinical Director, NIMH

Dr. Christy L. Ludlow, Research Speech Pathologist, Communicative Disorders
Program, NINCDS

Dr. Judith Rapoport, Chief, Section on Child Psychiatry, Laboratory of Clinical
Sciences, NIMH

Dr. Alan J. Zametkin, Clinical Associate, Section on Child Psychiatry,
Laboratory of Clinical Sciences, NIMH

Project Description:

Objectives: The purposes of this program are broad. An objective is to gain new knowledge of the central nervous system (CNS) of children and adolescents with special reference to maturational changes and neuropsychiatric disorders. Compared to the neurobiology known in adult neuropsychiatry, considerably less is known regarding the neuropsychiatric disorders of children. A particular focus of these studies has been the relationship between neurotransmitter change in hyperactive children (HAC) following compounds that have major actions on central neurotransmitter metabolism. The study of d-amphetamine (d-AMPH), a compound with clear and reliable effects in HAC, has been of particular interest, its pharmacokinetics, its effects on catecholamine and indoleamine metabolism and on behavior, and the interrelationships of these effects.

There have been a number of hypotheses relating catecholamine metabolism and hyperactivity in children. The possibility of an overly active catecholaminergic system was first advanced. Later, a functional deficiency in catecholamines in HAC was proposed with the greater focus on the possibility of a functional dopamine (DA) rather than norepinephrine (NE) deficiency, a hypothesis based on the following: 1) possible decreased functioning of the reward-system median forebrain bundle; 2) behavior in children with Von Economo's encephalitis resulting in a DA deficiency; 3) response to AMPH and its cyclized derivative, methylphenidate, both of which release NE and DA among other pharmacological actions; and 4) specificity of biochemical pharmacological interactions such as the proposed differences in the mode of action of d- and l-amphetamine. However, a number of later studies question whether the differential effects of d- and l-AMPH can be used to distinguish NE and DA metabolism. Other biochemical alterations, particularly involving serotonin (5-HT) have been proposed. More recently, alterations in phenylethylamine (PEA) have also been proposed. Dr. Michael H. Ebert and Dr. Judith L. Rapoport have provided overall collaboration and support for this multi-faceted project.

Methods Employed: An inpatient and day patient program for children and adolescents, involving selected overnight stays, is ongoing on an Inpatient Nursing Unit. Children who are hyperactive, aggressive and impulsive, and who have learning difficulties have been admitted in order to study a carefully defined population of HAC. Children and adolescents with other conditions have also been studied. Specific exclusion and inclusion criteria are employed.

All children are thoroughly evaluated by medical, psychiatric, and psychometric examinations with all routine and other indicated procedures and clinical laboratory studies. Children also receive a psycholinguistic examination in collaboration with NINCDS. Neurological examinations are scored carefully according to a rating scale (PANESS). Several clinical and behavioral rating instruments have been utilized.

Pharmacological compounds, both standard and those previously unused in children, are being studied. Serial plasma pharmacokinetic data are being generated for d-AMPH. These data are studied in conjunction with motor activity, behavior, cognition, speech, temperature, and cardiovascular response. Piribedil, a specific DA agonist, and L-DOPA have been given to HAC. Mianserin, a NE agonist, tryptophan (TP), a precursor of 5HT, clorgyline, a monoamine oxidase inhib-

itor (MAOI), and desipramine, a tricyclic antidepressant, have all been administered to HAC in clinical trials.

Motor activity is measured by an ambulatory activity monitor with solid state memory which measures individual motor movements via a pendulum acceleration system per unit of time and set at a desired sensitivity for the particular study. At any time the instrument can be read into a computer for a print-out. Behavioral changes in HAC are measured via Conners' Teachers' Rating Scale (CTRS). Cognition is measured by a continuous performance task (CPT) in which errors of omission and commission can be scored in terms of differing time intervals. Time intervals can also be increased or decreased in relationship to accuracy of response.

d-AMPH is measured by radioimmunoassay (RIA) and gas chromatograph mass spectrometry (GC-MS). Biochemical studies include 24-hour urine collection to study NE, 3-methoxy-4-hydroxyphenylglycol (MHPG), vanillylmandelic acid (VMA) and normetanephrine (NMN); DA, homovanillic acid (HVA), 3,4-dihydroxyphenylacetic acid (DOPAC) and 3-methoxytyramine (3-MT); tyramine (TRM) and parahydroxyphenylacetic acid (PHPA); and phenylethylamine (PEA) and phenylacetic acid (PAA) in collaboration with Dr. Alan J. Zametkin. Plasma pharmacokinetics of pharmacological compounds are being ascertained. Plasma NE, MHPG, NMN, and VMA changes as they relate to plasma d-AMPH levels have also been studied. Neurophysiological studies include routine and sleep EEG's and EMI scans when indicated. Averaged evoked response (AER) studies are being conducted as they relate to HAC in drug-free and treated conditions. Psycholinguistic changes, in collaboration with Dr. Christy L. Ludlow of NINCDS, are also studied in relation to d-AMPH, pibedil, and TP effects. Paired associate learning has also been assessed in different drug conditions. Chronic effects of d-AMPH (2 weeks) are being studied with regard to pharmacokinetics and clinical response, particularly in terms of evidence for tolerance or supersensitivity and effect on neurotransmitter metabolism, as manifested by changes in plasma NE, MHPG, HVA, and dopamine-beta-hydroxylase (DBH) and platelet 5HT and MAO. The effects of TP and valine and d-AMPH and placebo are being measured with regard to behavior, attention, rectal temperature, motor activity, and plasma amino acids and indoleamines.

Major Findings: Serial plasma pharmacokinetic data indicate that d-AMPH reaches a peak level in children within 3-4 hours of an initial dose; however, as much as 70-80% will remain in the serum at 5-6 h when behavioral effects have largely dissipated. Mean apparent elimination half-life is 6.8 ± 0.5 h. Test-retest studies of individuals indicate that both pharmacokinetic data and clinical response data are highly replicable. Sustained release capsules produce a slower rate of absorption and a more plateau-like, longer lasting peak level, but do not give a prolonged clinical response. Socially appropriate behavioral change and motor activity decrease is maximal at 1-3 h after administration of a single dose (0.5 mg/kg) of d-AMPH. Clinical changes may be related to a release of catecholamines and the subsequent depletion of their stores, replacement by a "false" neurotransmitter metabolite of AMPH, or to alteration in receptor sensitivity. Higher single doses (1.0 mg/kg) effect earlier similar clinical responses, but of less magnitude. Pibedil is safe but clinically ineffective in HAC. In a new study, d-AMPH has also been shown to have an anti-aggressive

effect in those HAC with a considerable degree of conduct disorder. In another current study, preliminary results indicate that neither TP nor valine (a neutral amino acid which competes with TP and inhibits its crossing the blood-brain barrier) results in behavioral response or basal temperature change after a single dose but attention span increase is similar to that observed following d-AMPH, while there are clear effects on plasma amino acids in the expected directions. This study was also designed such that the effects of the procedure itself could be accounted for. On the other hand, d-AMPH after a single dose appears to have no effect on serial plasma amino acids, 5HT, or 5-hydroxyindoleacetic acid (5HIAA) over a 6 h period. This preliminary finding could be quite important in that d-AMPH has been shown to have clear effects on central 5HT in animals. Another new study indicates that both plasma MHPG and HVA are affected acutely by single-dose d-AMPH in a non-pretreated child, but this biochemical response may not be the same following two weeks of d-AMPH. Further analysis of this study may have implications for receptor change.

Urine studies indicate that day and night excretion of MHPG and HVA are not different; however, d-AMPH after 8 and 14 days is associated with lower MHPG levels. Behavioral response may be associated with the decrement in MHPG. Urinary HVA is unchanged. These biochemical and behavioral findings have been replicated in a subsequent HAC group, not yet published, as well as extended to other metabolites of both NE and DA. TRM and PHPA excretion are also decreased and PEA excretion is markedly elevated following two weeks of d-AMPH. The TRM change may be associated with cardiovascular response and partially indicative of the change in PEA metabolism. More recent preliminary studies indicate a different pattern of metabolite response to methylphenidate, a drug which produces a behavioral effect similar to d-AMPH.

HAC are not different from normals with regard to plasma NE and DBH but do have significantly more neurological soft signs by PANESS examination. New item analysis data indicates the prevalence of varied soft signs and their rater reliability. Plasma NE correlates with anxiety ratings and changes both with regard to dose of d-AMPH and time following dose with higher doses of d-AMPH (1.0 mg/kg) giving strongest response at 1 hour and lower dose (0.5 mg/kg) giving strongest response at 3 hours. Elevated plasma NE is also associated with increases in blood pressure and pulse and is dose-related. In a new study, baseline plasma NE, measured prior to an early a.m. dose of d-AMPH, does not change after two weeks of d-AMPH vs. two weeks of placebo.

Preliminary results indicate a decreased platelet 5HT and increased platelet MAO in HAC vs. normals; however, platelet MAO also correlates positively and significantly with age.

With regard to pharmacological response, d-AMPH is effective and piribedil and L-DOPA are minimally so; TP produces responses similar to d-AMPH. HAC with higher levels of soft signs have more abnormal EEG's, more minor physical anomalies, lower full-scale I.Q.'s (WISC-R), and a greater number of errors on the Bender. Data from psycholinguistic evaluations indicates that HAC have impairments in certain auditory processing and language skills; furthermore, d-AMPH does not evoke pronounced effects with regard to language performance in HAC vs. normals; older and less hyperactive subjects showed the most improvement. Improvement in cognitive parameters was shown only in normals.

Significance to Mental Health Research: Though childhood neuropsychiatric disorders, and particularly HAC, have been considerably studied in the last few years, there are many diagnostic, psychopharmacological and psychobiological questions yet to be answered. Many studies in the past in child psychiatry have been related to psychological, psychodynamic issues. As regards HAC, obsessive-compulsive children, enuretics, Gilles de la Tourette's syndrome, anorexia nervosa, psychoses, and autism, an increased interest in psychopharmacology has emerged. Though methylphenidate and AMPH give positive responses in 80% of well diagnosed HAC, the pharmacokinetics and metabolism of these drugs have been studied only relatively recently. One avenue to ascertaining possible neuropathology in these conditions is to understand more clearly the mechanisms of action of those pharmacological compounds which effectively alter the clinical conditions under study. The relationship between such basic pharmacological knowledge and clinical effects has been under-studied in children in general. More importantly, for the future, basic biological factors in childhood neuropsychiatry which might elucidate the psychopharmacological responses are, at this point, only hypotheses. The degree to which these hypotheses are validated or refuted could play a significant role in our understanding of childhood neuropsychiatry.

Proposed Course of Project: The principal investigator, Dr. Brown, remains in the Office of the Chief, BPB, but is no longer administratively a part of the Section on Child Psychiatry newly transferred to the Laboratory of Clinical Science. The last active subjects from the present general project were completed during the spring of 1983. There is a considerable body of data yet to be analyzed but some of this is in preparation and in press and will be reported in the future. Dr. Robert M. Post, Chief, BPB, provides collaboration and support.

Publications:

Langer, D.H., Fletcher, J.C., Brown, G.L., Nee, L.E., and Smith, M.A.: Ethical considerations in psychological research in children. In Greenhill, L. and Shopsin, B. (Eds.): The Psychobiology of Childhood: Profiles of Current Issues. New York, Spectrum Publications, in press.

Brown, G.L., and Ebert, M.H.: Catecholamine metabolism and hyperactive children. In Lake, C.R. and Ziegler, M.G. (Eds.): Norepinephrine: Clinical Aspects. Baltimore, Williams & Wilkins, in press.

Ludlow, C., Cudshy, E., Bassich, C., and Brown, G.L.: The auditory processing skills of hyperactive, language impaired and reading disabled boys. In Katz, J. and Lasky, E.Z. (Eds.): Central Auditory Processing Disorders: Problems of Speech, Language, and Learning. Baltimore, University Park Press, in press.

Brown, G.L., Ebert, M.H., Minichiello, M.D.: Biochemical and pharmacological aspects of attention deficit disorder. In Bloomingdale, L.M. (Ed.): Attention Deficit Disorder. New York, Spectrum Publications, in press.

Amery, B., Minichiello, M.D., Brown, G.L.: Aggression in hyperactive boys: Response to d-amphetamine. J. Am. Acad. Child Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00034-13 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychological and Physiological Correlates of Average Evoked Response		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. S. Buchsbaum, M.D., Chief, Sect. on Clin. Psychophysiology, BPB, NIMH		
COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology George Washington University, Washington, D.C.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Clinical Psychophysiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="height: 400px; border: 1px solid black; margin-top: 10px;"></div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00035-11 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Psychopharmacological Correlates of the Averaged Evoked Response		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. S. Buchsbaum, M.D., Chief, Sect. on Clin. Psychophysiology, BPB, NIMH		
COOPERATING UNITS (if any) Clinical Psychobiology Branch; Clinical Neuropharmacology Branch; Lab. of Psychology & Psychopathology; DCBR, NIMH. Dept. of Clinical Psychology, Univ. of Maryland; Dept. of Psychiatry, Univ. of Minnesota		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Clinical Psychophysiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 0.6	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been incorporated into Project #Z01 MH 00507-01 LPP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00036-09 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Individual Differences in Sleep and AER		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> M. S. Buchsbaum, M.D., Chief, Sect. on Clin. Psychophysiology, BPB, NIMH		
COOPERATING UNITS <i>(if any)</i> Clinical Psychopharmacology, St. Elizabeth's Hospital, Washington, D.C.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Clinical Psychophysiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.1	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <div style="text-align: center; padding-top: 50px;"> <p>This project has been discontinued.</p> </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00039-09 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Sensory Thresholds and Averaged Evoked Responses		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> M. S. Buchsbaum, M.D., Chief, Sect. on Clin. Psychophysiology, BPB, NIMH		
COOPERATING UNITS <i>(if any)</i> Clinical Neuropharmacology Branch; Clinical Psychobiology Branch, DCBR, NIMH. Dept. of Psychiatry, Univ. of Tennessee, Memphis, Tennessee.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Clinical Psychophysiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> This project has been incorporated into Project #Z01 MH 00507-01 LPP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00041-03 BP
PERIOD COVERED October 1, 1983 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Simultaneous Electroencephalography & Cerebral Glucography with PET in Normals & Schizophrenics		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> M. S. Buchsbaum, M.D., Chief, Section on Clinical Psychophysiology, BPB, NIMH		
COOPERATING UNITS <i>(if any)</i> Clinical Center, NIH; LPP & LCM, NIMH; Univ. of Lund, Sweden		
LAB/BRANCH Biological Psychiatry Branch, NIMH		
SECTION Section on Clinical Psychophysiology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> This project has been incorporated into Project #Z01 MH 00507-01 LPP.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00070-10 BP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychological and Biological Interactions in the Mood and Anxiety Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
Robert M. Post, M.D.	Chief	BP NIMH
COOPERATING UNITS (if any) APB, CNB, CPB, LCM, LCS, LPP, RSB, IRP, NIMH; DPCBR, NIAAA; PDS, NIH; USUHS, Dept of Def.; Univs. of CA., Chicago, VA.; VA Med. Cntr, Bronx; San Diego VA Med Cntr.; Tufts Univ.; Univ. Hosp., Munich; Wellesley Hosp., Toronto; Thos. Jefferson Univ., Walter Reed Med. Cntr.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychobiology		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, MD 20205		
TOTAL MANYEARS: 12.0	PROFESSIONAL: 6.0	OTHER: 6.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Patients suffering from manic, depressive, schizoaffective, and anxiety-related disorders are longitudinally evaluated and treated. <u>Double-blind, placebo-controlled clinical trials</u> are employed. Classical <u>neurotransmitters</u> and their metabolites, as well as <u>hormones</u> and <u>peptides</u> that have been implicated in the regulation of mood and behavior, are measured in blood and CSF of patients to assess their relationship to normal and pathological behavior. Alterations in cognitive function, neurophysiology, and biochemistry are explored in relationship to predictors and mechanisms underlying clinical response to <u>anticonvulsants</u>, <u>dopaminergic</u> and <u>noradrenergic receptor agonists</u>, the paradoxical therapeutic effects of <u>sleep deprivation</u> in depression, and related treatments of mood and anxiety disorders. Ongoing clinical trials with <u>carbamazepine</u> indicate it may be a useful alternative to lithium carbonate for the acute and prophylactic treatment of manic-depressive illness. Its mechanisms of action in affective illness are being explored. Animal models of <u>electrical kindling</u> and <u>stimulant-induced behavioral sensitization</u> are explored in order to examine mechanisms underlying progressive changes in behavioral pathology. </p>		

COLLABORATORS:

Dr. T. Colburn, Research Services Branch, NIMH
Dr. W. Mendelson, Clinical Psychobiology Branch, NIMH
W. Duncan, Clinical Psychobiology Branch, NIMH
Dr. H. Holcomb, Biological Psychiatry Branch, NIMH
Dr. R. Cohen, Clinical Neuropharmacology Branch, NIMH
Dr. L. DeLisi, Biological Psychiatry Branch, NIMH
Dr. M.S. Buchsbaum, Dept. of Psychiatry, U. of California, Irvine
Dr. T.W. Uhde, Biological Psychiatry Branch, NIMH
Dr. L. Siever, VA Medical Center, Bronx, N.Y.
Dr. D.L. Murphy, Clinical Neuropharmacology Branch, NIMH
Dr. D.C. Jimerson, Laboratory of Clinical Science, NIMH
Dr. F.K. Goodwin, Intramural Research Program, NIMH
Dr. P.W. Gold, Biological Psychiatry Branch, NIMH
Dr. M.H. Ebert, Intramural Research Program, NIMH
Dr. M. Linnoila, Div. of Intramural Clinical and Biological Research, NIAAA
Dr. W.H. Berrettini, Biological Psychiatry Branch, NIMH
Dr. H. Weingartner, Laboratory of Psychology and Psychopathology, NIMH
Dr. D.R. Rubinow, Biological Psychiatry Branch, NIMH
Dr. L. Bierer, Biological Psychiatry Branch, NIMH
Dr. C.H. Kellner, Biological Psychiatry Branch, NIMH
Dr. B. Vittone, Biological Psychiatry Branch, NIMH
Dr. R. Coppola, Laboratory of Psychology and Psychopathology, NIMH
Dr. R. Cowdry, Intramural Research Program, NIMH
Dr. S. Weiss, Biological Psychiatry Branch, NIMH
Dr. A. Pert, Biological Psychiatry Branch, NIMH
Dr. T. Insel, Clinical Neuropharmacology Branch, NIMH
Dr. P. Marangos, Clinical Psychobiology Branch, NIMH
Dr. J. Patel, Clinical Psychobiology Branch, NIMH
Dr. D. Jacobowitz, Laboratory of Clinical Science, NIMH

Dr. C. Kennedy, Laboratory of Cerebral Metabolism, NIMH
Dr. L. Sokoloff, Laboratory of Cerebral Metabolism, NIMH
Dr. J. C. Gillin, Dept. of Psychiatry, San Diego VA Medical Center, CA.
Dr. D.C. Chatterji, Pharmaceutical Development Service, NIH
Dr. R.F. Greene, Pharmaceutical Development Service, NIH
E. Gordon, Laboratory of Clinical Science, NIMH
Dr. C. R. Lake, Uniformed Services Univ. of the Health Sciences, Dept. of Defense
Dr. D. Hommer, Neurosciences Branch, NIMH
Dr. S. Reichlin, Division of Endocrinology, Tufts University
Dr. J.C. Ballenger, Dept. of Behav. Med. and Psychiatry, U. of VA Med. Center
Dr. D. Naber, University Clinic, University Hospital, Munich, Germany
Dr. D. Pickar, Neurosciences Branch, NIMH
Dr. R. Adamec, Limbic Research Center, Wellesley Hospital, Toronto
Dr. F. Putnam, Adult Psychiatry Branch, NIMH
Dr. R.G. Savard, Biological Psychiatry Branch, NIMH
Dr. T. Hare, Jefferson Medical College, Thomas Jefferson University
Dr. E. Gershon, Biological Psychiatry Branch, NIMH
Dr. G.L. Brown, Biological Psychiatry Branch, NIMH
Dr. P. Roy-Byrne, Biological Psychiatry Branch, NIMH
Dr. W.E. Bunney, Jr., Dept. of Psychiatry, Univ. of California, Irvine
Dr. G. Robertson, Dept. of Medicine, U. of Chicago
Dr. D. Fisher, Dept. of Medicine, University of Chicago
T. Porcu, Biological Psychiatry Branch, NIMH
D. Davis, Biological Psychiatry Branch, NIMH
Dr. E.K. Silberman, Liaison Psychiatry, Walter Reed Army Medical Center
Dr. J.-P. Boulenger, Biological Psychiatry Branch, NIMH
Dr. K. Zander, Biological Psychiatry Branch, NIMH
N. Contel, Biological Psychiatry Branch, NIMH

I. Project Description

A. Objectives

This project is engaged in the multidisciplinary longitudinal study and treatment of patients with a spectrum of acute and chronic psychoses, particularly involving mood and anxiety disorders. Both investigative and treatment approaches focus on the elucidation of psychological and biological phenomena and their complex interaction.

B. Methods Employed

1. Subjects

a. Subjects who meet Research Diagnostic Criteria (RDC) for manic-depressive or schizoaffective illness or the more recent DSM III criteria for a spectrum of mood disorders are admitted to the 3-West Clinical Research Unit, Section on Psychobiology. Patients with anxiety and panic anxiety are also admitted to the unit under other protocols (see Project Z01 MH 00071-03 BP).

b. Normal volunteers are also admitted to the unit to provide control data for specific studies in patients and to assess clinical and biological interrelationships in normal as well as patient populations. Volunteers complete an extensive battery of biochemical, psychological, and physiological tests including lumbar punctures (LP's). LP's are performed at 9 AM and 9 PM to study alterations in circadian rhythms in patients and volunteers.

2. Psychological and Biological Evaluation

a. Behavior and Cognition: During an initial drug-free interval patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation, including EEG-monitored sleep, averaged evoked potentials, and a variety of cognitive tests. These include the Halstead Category Test, a psychosensory questionnaire, a neuropsychological profile using the Luria Test and an extensive battery of tests developed in collaboration with K. Squillace and A.F. Mirsky of the Laboratory of Psychology and Psychopathology.

Longitudinal behavioral data are collected in a double-blind fashion utilizing twice-daily global ratings by trained nursing observers. Patients also complete twice-daily ratings of mood and side effects in order to examine diurnal variation. Using the same double-blind methodology, nurses also evaluate patients on a modified Brief Psychiatric Rating Scale (BPRS) three times weekly.

b. Life Chart Methodology: A life chart technique has been developed to plot the number and severity of affective episodes and the interval between episodes so that the longitudinal development, recurrence, and progression of the illness can be accurately quantitated and illustrated. This technique is an important clinical as well as research tool for assessing the efficacy of treatment interventions.

c. Physiology: Motor activity is measured continuously at 15-minute intervals with a miniaturized activity monitor developed by Dr. T. Colburn. EEG-monitored sleep is studied in collaboration with Dr. W. Mendelson and W. Duncan. In collaboration with Drs. Henry Holcomb and M.S. Buchsbaum, 16-channel EEG's, averaged evoked responses, and studies of hemispherical laterality and psychophysiological pain are conducted.

d. Functional Anatomy: In addition to computerized axial tomography (CAT-scan) evaluation of our patients for possible cerebral pathology, studies have been initiated in collaboration with Drs. Robert Cohen, Lynn DeLisi and M.S. Buchsbaum and associates to study regional functional activity of the brain using (18F) fludeoxyglucose.

e. An alpha-Adrenergic Agonist, Clonidine: Clonidine is administered intravenously to depressed and anxious patients and volunteers in order to assess clinical, physiological, and neuroendocrine responses to this alpha-adrenergic agonist (with Drs. T.W. Uhde, L. Siever and D.L. Murphy).

f. alpha-Adrenergic Receptors: In collaboration with Dr. M. Kafka, platelet alpha receptor function, as well as prostaglandin-stimulated increases in cyclic-AMP and their inhibition by norepinephrine, are assessed in normal volunteers and patients with mood and anxiety disorders.

g. Urinary Free and Plasma Cortisol; Dexamethasone Suppression; CRF Stimulation; and Lymphocyte Glucocorticoid Binding: Basal 24-hour urinary free cortisol is measured during depressed and manic states in medication-free conditions and during treatment. A detailed evaluation of the pituitary-adrenal axis is conducted by Dr. D.R. Rubinow in patients with affective illness and anxiety disorders. Plasma cortisol is measured under basal conditions and following the dexamethasone suppression test. Glucocorticoid receptor binding on lymphocytes is assessed in collaboration with Dr. D.C. Jimerson. Hormonal response to CRF before and during treatment with carbamazepine is studied with Dr. P.W. Gold (see below).

h. Cerebrospinal Fluid (CSF) and Plasma Studies: Plasma and CSF studies comprise an important area of biological evaluation of classical neurotransmitters and their amines as well as the newly discovered peptide substances in normal volunteers and in patients during ill and well intervals. These studies are conducted in collaboration with Drs. F.K. Goodwin, P.W. Gold, D.C. Jimerson, M.H. Ebert and M. Linnoila, as well as many investigators within and outside of NIMH with specialized techniques for measurement of specific peptide hormones. Several measures of GABA metabolism are obtained in plasma or CSF of affectively ill patients or controls in collaboration with Dr. W. Berrettini.

i. Oxytocin and Vasopressin: In collaboration with Drs. H. Weingartner, P.W. Gold, and D.R. Rubinow, infusions of these peptides are utilized to assess effects on memory, mood, and endocrine function in affectively ill patients and normal volunteers.

j. Adrenergic Mechanisms of Cortisol Hypersecretion: Propranolol and thymoxamine are administered to affectively ill patients and controls to assess whether cortisol hypersecretion is under the regulatory control of the adrenergic system (in collaboration with L. Bierer and D. Jimerson).

k. Corticotropic Releasing Hormone: CRF is studied in collaboration with Dr. P.W. Gold.

l. Procaine Activation: Procaine, an agent which activates limbic system structures with some selectivity, is administered intravenously in graded

doses to affectively ill, borderline, and normal subjects to assess possible altered behavioral, electrophysiological, or biochemical responsivity in this system. Collaborators include C. Kellner, B. Vittone, R. Coppola and R. Cowdry.

m. Platelet Vasopressin Receptors: AVP receptors on platelets are studied in collaboration with Dr. W. Berrettini.

3. Treatment

a. Psychotherapeutic: Treatment and evaluation are conducted in individual and group therapy, and ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment: Both routine and experimental compounds are evaluated during double-blind clinical trials. The routinely used drugs include tricyclic antidepressants, lithium carbonate, monoamine oxidase inhibitors, and neuroleptics. These agents are utilized not only because of their clinical efficacy, but as well to further understand their mechanisms of action and possible interaction with the pathophysiology of the illness.

c. Experimental Compounds: The anticonvulsant carbamazepine has been introduced as a new treatment for manic and depressive illness and is evaluated for its acute and prophylactic efficacy. Diphenylhydantoin and valproic acid are two other anticonvulsant agents also being studied in selected patients to assess the specificity of the positive psychotropic effects of carbamazepine in relation to other anticonvulsants with different spectrums of clinical efficacy.

d. Receptor Agonists: Clonidine, in addition to acute intravenous studies, is administered during clinical trials to assess the clinical efficacy of alterations in adrenergic functioning in anxiety and affective illness.

e. Peptide Strategies: In addition to acute challenges with oxytocin and vasopressin, clinical trials have been conducted in collaboration with Dr. P.W. Gold of a vasopressin analog, DDAVP, in affective illness.

f. Sleep Deprivation: The paradoxical antidepressant effects of one night's sleep deprivation in depressed patients are explored both to develop a model for further understanding the rapid onset and offset of a non-pharmacologically-induced mood improvement and to assess its therapeutic potential.

4. Animal Models.

A rodent and primate behavioral pharmacology laboratory is maintained in collaboration with S. Weiss, A. Pert, and T. Insel to develop new research techniques in several areas. The longitudinal evolution of behavioral pathology is assessed using a number of paradigms including: 1) electrophysiological kindling; 2) pharmacological kindling; 3) behavioral sensitization to psychomotor stimulants and related dopaminergic agonist compounds; and 4) the evaluation of stress sensitization and learned helplessness and their possible underlying neural substrates. Physiological and biochemical changes, particularly alterations in receptor binding, are studied in collaboration with A. Pert, P. Marangos, J. Patel and D. Jacobowitz. ¹⁴C-2-deoxyglucose studies have been conducted utilizing pharmacological kindling with lidocaine in collaboration with Drs. C. Kennedy, L. Sokoloff, and associates. The role of seizures in the development of behavioral pathology is studied utilizing a variety of seizure models, behavioral assessments, and anticonvulsant compounds.

C. Major Findings

1. Carbamazepine: A New Treatment for Manic-Depressive Illness

Dr. Post received the Foundation's Fund Prize Award for Research in Psychiatry given by the American Psychiatric Association for his work on carbamazepine and its theoretical underpinnings.

a. Introduction: Several empirical observations and theoretical perspectives led to our initiation of the first double-blind, placebo-controlled clinical trials of carbamazepine in mania and depression in the United States. There had been persistent reports of positive effects on mood and behavior in epileptic patients treated with carbamazepine. Carbamazepine, both clinically and in experimental models such as kindling, is the most effective anticonvulsant against temporal lobe-limbic seizures. Temporal lobe and limbic structures have long been hypothesized to be importantly involved in the modulation of normal and pathological affect. As such, an agent which stabilized abnormal excitability in this area of brain might be expected to have stabilizing effects on emotional function. Moreover, preliminary data from open clinical trials in Japan suggested that carbamazepine might be effective in manic-depressive patients when it was added to previously ineffective drug regimens.

b. Acute Antimanic Efficacy: In collaboration with Tom Uhde we continue to document unequivocal evidence of the efficacy of carbamazepine in the acute treatment of manic episodes. It is noteworthy that this occurs in many patients who were previously nonresponsive to lithium carbonate, the more traditional agent for the treatment of affective illness. Evidence of carbamazepine response has been documented during an "off-on-off-on" design, where carbamazepine and placebo are administered in an alternate fashion, with nurses blind to this clinical trial. We have noted repeated clinical improvement during carbamazepine treatment and exacerbation during placebo substitution.

c. Acute Antidepressant Efficacy: Sixteen of the first 31 patients have shown evidence of clinical response to carbamazepine. In some instances marked clinical improvement was observed in previous drug nonresponders but in most the improvement was mild to moderate. Relapses following placebo substitution were not as consistently observed in the depressive phase as they were in the use of carbamazepine for the treatment of mania. Therefore, like lithium, carbamazepine appears to have clear antimanic but less well documented acute antidepressant properties. Degree of antidepressant response correlated with CSF and plasma levels of carbamazepine-10,11-epoxide. Patients with more severe depression responded better to carbamazepine than those with less severe initial ratings of depression on placebo. Family history of affective illness did not predict response.

d. Prophylactic Efficacy of Carbamazepine: Seven patients have been followed for a mean of 1.7 years on carbamazepine in either a double-blind or an open fashion. In these lithium-nonresponsive, rapidly cycling manic-depressive patients carbamazepine decreased the mean number of affective episodes from 16.4 ± 5.7 in the year prior to carbamazepine treatment to 5.6 ± 2.3 episodes/year on the drug. The severity and duration of episodes when they did occur were also reduced on carbamazepine. Discontinuation of the drug resulted in relapses in five of six patients, further indicating that improvement was related to carbamazepine and not to spontaneous improvement in the course of illness.

e. Side Effects: The drug is well tolerated in the majority of patients, with mild and clinically insignificant decreases in white count observed in the majority of patients. Mild decreases in serum sodium are also observed, as documented by T. Uhde. Sedation and dizziness are dose-related and tend not to occur with slow increases in dose. Analysis of self-ratings of side effects indicates that depressed patients experience a moderate incidence of apparent drug-related side effects while medication-free on placebo, and while on carbamazepine do not show an increase in any of these "side effects".

While daytime sedation is not a problem, substantial improvement in sleep has been noted in half-hour sleep checks by nurses blind to active carbamazepine administration. In the first 27 depressed patients, sleep significantly increased ($p < .001$) during the first week of carbamazepine. This often preceded clinical improvement in depressed mood and was maintained during the clinical trial. Similarly, in the first 11 manic patients studied, sleep almost doubled in the first week of carbamazepine administration ($p < .001$). EEG studies in collaboration with Dr. W. Mendelsohn, Dr. J.C. Gillin, and W. Duncan will document the stages of EEG-monitored sleep that are affected.

f. Plasma and CSF Levels of Carbamazepine and Its -10,11-Epoxy Metabolite: Spinal fluid levels of carbamazepine and its 10,11-epoxide metabolite were measured in 18 affectively ill patients. These studies were performed in collaboration with Drs. T.W. Uhde, D.C. Chatterji, and R.F. Greene. Mean CSF carbamazepine was 2.06 ± 0.10 ug/ml, while the epoxide was $0.91 \pm .09$ ug/ml or 44% of the concentration of the parent compound. Carbamazepine levels in plasma or in CSF (a measure of free carbamazepine) were not significantly related to degree of clinical antidepressant or antimanic response.

However, CSF levels of carbamazepine-10,11-epoxide were significantly correlated with the degree of clinical response ($r = .67$, $p < .005$). Similar relationships were also observed in plasma where the epoxide, but not carbamazepine itself, was correlated with the degree of clinical response. These data suggest that in those patients treated with an average of 1000 mg/day of carbamazepine, achieving plasma levels between 6 and 12 ug/ml, there is not a close relationship between carbamazepine levels and clinical response. Similar observations have been made in the neurological literature in relationship to anticonvulsant efficacy. However, our data suggest the possibility that the -10,11-epoxide metabolite, which we and others have noted to have anticonvulsant effects in animals, may also possess active psychotropic properties in man.

g. Comparison With Other Anticonvulsants: Clinical trials have been initiated to examine the relative efficacy of carbamazepine in comparison to other anticonvulsants such as phenytoin and valproic acid. In the first patient to complete a double-blind crossover design, no evidence of clinical improvement was observed with phenytoin or valproic acid, while the patient was an unequivocal carbamazepine responder. These data suggest the possibility that biochemical or physiological properties peculiar to carbamazepine may, at least in this patient, be important to its psychotropic properties rather than relating to generalized anticonvulsant effects. Emrich and associates in Europe have, however, reported the successful use of valproic acid in a small number of lithium-resistant manic-depressive patients. Further clinical trials of these agents are indicated.

Although carbamazepine is a highly effective anticonvulsant, it is also useful in the treatment of a variety of paroxysmal pain syndromes which clearly do not involve an ictal process. Thus, the efficacy of carbamazepine does not imply that subclinical seizures are the underlying pathophysiological mechanism in patients with affective illness. However, the properties mediating carbamazepine's anticonvulsant effects may nonetheless be related to its psychotropic properties. The clinical utility of the anticonvulsant carbamazepine raises the paradox of why the major motor seizures of electroconvulsive therapy are among the most effective treatments for acute manic and depressive illness. As detailed below, we have documented that electroconvulsive seizures in the rat are paradoxically anticonvulsant to amygdala-kindled seizures. These data raise the possibility that common biochemical and physiological mechanisms of electroconvulsive therapy in man and the anticonvulsant carbamazepine on limbic system excitability could be related to their profile of therapeutic efficacy in both phases of affective illness.

h. Studies of Carbamazepine's Mechanism of Action:

1) Effects on Classical Neurotransmitters and Modulators: Evidence in laboratory animals (Purdy et al.) suggests that carbamazepine blocks the reuptake of norepinephrine (NE) but also inhibits stimulated-induced release. We have observed, in collaboration with Dr. D.C. Jimerson and E. Gordon, that carbamazepine treatment significantly reduces the NE metabolite 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) in CSF of patients with affective illness. CSF NE itself, measured in collaboration with Dr. C.R. Lake, is not significantly altered in the depressed patients; however, the elevated levels of CSF NE in mania are decreased by carbamazepine. Noradrenergic effects of carbamazepine have indirectly been linked to its anticonvulsant properties. CSF 5-hydroxyindoleacetic acid (5HIAA) is also not significantly affected by carbamazepine.

The possible dopaminergic effects of carbamazepine are of considerable interest, but presently remain to be further clarified. There is substantial indirect evidence that carbamazepine does not act as a classical neuroleptic. It does not appear to block cocaine- or amphetamine-induced hyperactivity or stereotypy and does not raise HVA levels in rat brain or in the spinal fluid of our patients with affective illness, as do the classical neuroleptic treatments. Moreover, it has not been associated with the development of parkinsonian side effects or with the syndrome of tardive dyskinesia as have the neuroleptic drugs. Interestingly, carbamazepine produces slight but statistically significant increases in serum prolactin in contrast to the major increases in prolactin achieved by traditional antipsychotic agents. It does not displace ³H-spiroperidol binding (Marangos et al., 1983) or increase firing of dopamine neurons in the substantia nigra (P. Clark and D. Hommer). These data indicate that carbamazepine has differential effects on dopaminergic mechanisms that could be important to its profile of effects and side effects in epilepsy and affective illness.

Alterations in GABA have been postulated both in affective illness (see below) as well as in the seizure disorders. Carbamazepine has been reported to decrease the turnover of GABA in animal studies, although brain levels are not altered by the drug. This is consistent with our data indicating that CSF GABA levels are not significantly decreased during treatment with carbamazepine compared to baseline levels.

Effects of carbamazepine on central and "peripheral" benzodiazepine receptors have been studied with biochemical techniques (Marangos et al.) and electrophysiologically in the amygdala kindling model. Carbamazepine binds poorly to the central site (^3H -diazepam or ^3H -BCCE), but more potently at the Ro 5-4864 (peripheral) site. In parallel, the antagonists, Ro 15-1788 and CGS-8216, which block actions at the central site, are ineffective in reversing the anticonvulsant effects of carbamazepine in amygdala kindling, but Ro 5-4864 does reverse carbamazepine's anticonvulsant effects. Taken together, these biochemical and electrophysiological data suggest the possibility that carbamazepine may have physiologically relevant effects on the Ro 5-4864 site in brain.

Carbamazepine is potent in displacing binding of several adenosine receptor ligands (Marangos et al.). However, the anticonvulsant efficacy of 12 carbamazepine analogs on ECS seizures in mice does not correlate with the ability to displace either adenosine agonist or antagonist binding. Several adenosine agonists and antagonists also do not affect carbamazepine's effects on amygdala kindling. These data suggest that carbamazepine's potent effects on amygdala kindling may be related to a property of the drug other than its adenosine effects.

Cyclic nucleotides (cyclic-AMP and cyclic-GMP) have been postulated to play an important role in the therapeutic effects of a variety of psychotropic and anticonvulsant agents. While carbamazepine did not affect basal levels of these in the CSF of our affectively ill patients, probenecid-induced accumulations tended to be significantly reduced.

2) Carbamazepine's Effects on Endocrine and Peptide Systems:

Carbamazepine significantly decreased somatostatin measured in CSF of affectively ill patients in collaboration with Drs. D.R. Rubinow, P.W. Gold, and S. Reichlin. These findings are of interest in relationship to the report of long-lasting increases in brain somatostatin following amygdala kindling seizures and the findings of low CSF somatostatin in depressed patients.

Carbamazepine's effects on vasopressin are noteworthy from both a clinical and theoretical perspective. In contrast to lithium carbonate which produces the diabetes insipidus syndrome, carbamazepine has been used to treat diabetes insipidus. It has antidiuretic properties which are manifest by its effects in producing mild hyponatremia (T.W. Uhde). During carbamazepine treatment, decreased endogenous vasopressin is secreted in response to a hypertonic saline load, also consistent with an agonist role in this system (P.W. Gold and J.C. Ballenger). These findings are opposite those observed during lithium carbonate treatment. Dr. W.H. Berrettini has documented that carbamazepine is the one psychotropic drug tested to date that displaces ^{125}I -arginine vasopressin binding from platelets, further suggesting that carbamazepine may have direct effects at the vasopressin receptor. The relationship of carbamazepine's antidiuretic effects to possible alterations in mood and cognition remain to be explored.

The effects of carbamazepine on cortisol are noteworthy from several perspectives. Rubinow and associates have found that carbamazepine induces escape from dexamethasone suppression, even in depressed patients who are showing clinical improvement. It is unlikely that the effects of carbamazepine on dexamethasone metabolism entirely account for escape from dexamethasone suppression, as urinary free cortisol is also increased in some populations studied on carbamazepine. Carbama-

zepine may thus be affecting regulation of the pituitary-adrenal axis directly or through its effects on higher neural substrates in the limbic system or elsewhere. The carbamazepine-induced decrease in somatostatin described above could account for the effects on cortisol, as somatostatin has been shown by others to inhibit CRF stimulation of ACTH. The vasopressin agonist properties of carbamazepine could also account for the cortisol effects as vasopressin appears to stimulate cortisol secretion in man (Rubinow et al.).

Continued study of carbamazepine's biochemical effects, either alone or in comparison and contrast to lithium carbonate, may ultimately prove useful not only in further understanding its mechanism of action in affective illness, but also in helping to understand substrates underlying the affective disorders. The possible effects of carbamazepine on endogenous opiate systems are of interest in relation to the efficacy of carbamazepine in pain syndromes and the fact that it potentiates opiate-induced running activity in mice. There was no significant effect of carbamazepine on CSF opiate binding activity in 17 affectively ill patients, studied in collaboration with Drs. D. Naber, D. Pickar, and associates. Discrete effects of carbamazepine on regional opioid sub-systems in brain remain to be ruled out, however.

3) Procaine Activation as a Predictor of Carbamazepine Response?

The local anaesthetic procaine has been shown to alter limbic system electrophysiological activity in animals. In man it produces a variety of psychosensory distortions that are highly similar to those produced by direct electrical stimulation of amygdala or hippocampus (in the studies of Gloor et al. and Dalgren et al.). In collaboration with R. Adamec, who is analyzing omega band (31 to 50 Hz) activity in anterior leads which are thought to reflect amygdala activity, and R. Coppola who is analyzing topographic electrical activity, our group are studying dose-related behavioral, physiological, and endocrine responses to i.v. procaine (maximum dose, 2.3 mg/kg). This investigational group includes C. Kellner, B. Vittone, F. Putnam, R. Cowdry and R. Adamec.

Dose-related cognitive and psychosensory alterations have been noted and mood alteration consisting of euphoria or dysphoria has been observed. Vivid recall of experientially immediate memories and images occurred less often. The sensory alterations were accompanied by dose-related theta activation on the computer-analyzed EEG. Procaine increased secretion of plasma beta-endorphin, ACTH, and cortisol, suggesting the possibility that it may affect limbic centers controlling release of CRF.

Whether these cognitive, affective, electrophysiological, or endocrine responses, perhaps reflective of limbic system responsivity in man, will predict subsequent response to carbamazepine in affectively ill patients is also a major question addressed by this study.

2. Approaches to Neurotransmitter Receptor Dysfunction in Affective Illness

a. Noradrenergic Systems: alpha-Adrenergic receptors have been measured on platelets of drug-free patients with affective disorders and normal control subjects in collaboration with Dr. M. Kafka. The number of receptors measured by tritiated dihydroergocryptine was significantly increased in patients, while noradrenergic inhibition of prostaglandin PGE₁-stimulated cyclic-AMP production was

significantly reduced. Parallel findings have been observed in panic-anxious patients (Uhde et al.). In contrast to these measurements in platelets, endocrine responses have been blunted following the acute intravenous administration of the alpha-2 receptor agonist clonidine, in collaboration with Drs. T.W. Uhde, L.J. Siever, and D.L. Murphy.

Drs. Uhde and Siever received the prestigious A.E. Bennett Prize for clinical research from the Society for Biological Psychiatry for this work and that described below.

Consistent with its effects on decreasing firing of the noradrenergic locus coeruleus in animals, clonidine acutely decreased plasma NE and MHPG, measured in collaboration with Drs. C.R. Lake and D.C. Jimerson. Clonidine was associated with antianxiety effects measured on the Spielberger Rating Scale in depressed and anxious patients. No significant effects on anxiety were observed following placebo administration or in the normal volunteer subjects. Clonidine's effects are consistent with the observations that CSF NE (R. Lake) and CSF MHPG (D.C. Jimerson) may be slightly elevated in some depressed patients (possibly those with greater anxiety) compared to normal volunteers. However, CSF NE is markedly increased in manic patients compared to either of the other patient or control populations. In normal volunteers plasma MHPG, measured by Dr. Jimerson, was observed to correlate significantly and negatively with severity of depression, hypochondriasis, and psychasthenia scales measured on the Minnesota Multiphasic Personality Inventory (MMPI). The data raise the possibility that noradrenergic mechanisms may be associated with the normal as well as pathological range of affective function. Measurements of noradrenergic function in blood, urine, and spinal fluid of these affectively ill patients, in collaboration with D.C. Jimerson and J.C. Ballenger, are also helpful in clarifying the role of interrelationships between noradrenergic measures in different body fluids.

In addition to the state-related alterations in noradrenergic function, we have been interested in assessing the relationship of this system to the longitudinal course of affective illness, as assessed by life chart methodology. We have observed that those patients with higher CSF NE had greater numbers of episodes in the year prior to NIMH admission ($r = .61$, $p < .05$). Those with higher CSF NE during the depressive state experienced greater numbers of weeks ill in the year prior to NIMH admission ($r = .76$, $p < .001$). We have also followed a group of patients to assess social functioning an average of 3.5 years following discharge from NIMH (unpublished data with Dr. R.J. Savard). Patients who had poor social functioning measured in the social and leisure activity subscale of the Social Adjustment Scale had higher CSF VMA ($r = .66$, $p < .02$, $n = 13$) and higher CSF NE ($r = .80$, $p < .005$, $n = 11$). These findings, taken together, suggest that increases in noradrenergic function measured during an acute episode of depression may be positively related to the longitudinal course of affective illness and to greater frequency of cycling as well as poorer prognosis variables. These are among the first observations of biological correlates associated with the longitudinal, rather than acute state-related, course of affective illness.

b. GABA: In collaboration with Dr. W.H. Berrettini, we have recently reviewed the literature on possible alterations in GABA-ergic mechanisms in affective illness. Indirect pharmacological data support a possible role of GABA in affective illness. Moreover, direct measurements of GABA in plasma and CSF provide

some evidence of disturbed GABA function. Plasma GABA, measured in collaboration with T. Hare, was significantly lower in euthymic medication-free patients compared to normal controls. Three or four studies in the literature have reported low CSF GABA in depression compared to control groups. We have observed significantly lower levels in individuals studied longitudinally during depressed compared to manic phases of the illness. Dr. Berrettini, in conjunction with Dr. E. Gershon, has collected further evidence that GABA may, in part, be regulated at a genetic level as well as fluctuating in a state-related fashion. Plasma GABA levels were significantly correlated in identical twin pairs. Dr. Berrettini has also measured GABA transaminase (GABA-T) and found this enzyme to be significantly lower in affectively ill patients compared to normal volunteers. These studies, suggesting possible GABA alterations in affective illness, are of interest in relation to recent reports that GABA agonists may have antidepressant effects, and that several agents reported to be effective in the treatment of recurrent affective illness (electroconvulsive therapy, carbamazepine, and valproic acid) all decrease GABA turnover. Dr. Berrettini has also observed that lithium carbonate may alter GABA function by blocking GABA uptake.

c. Dopamine: Indirect biochemical, pharmacological, and endocrine data continue to suggest a role for dopamine in some aspects of affective illness. Dopamine and its metabolite HVA and DOPAC are studied in collaboration with Dr. M. Linnoila in depressed, manic and euthymic patients and controls. The relationship to the longitudinal course of affective illness is being assessed.

d. Serotonin: Dr. G.L. Brown has found that low 5HIAA in CSF of several patient populations is correlated with aggressivity directed externally or internally (suicidality). Dr. Roy-Byrne has found that this relationship is not observed in bipolar depressed patients, however. In hyperactive children Dr. Brown has found that the serotonin precursor tryptophan alters amino acid interrelationships and has clinical effects similar to that of amphetamine. Studies of 5HIAA in relation to aggression, suicidality, and the longitudinal course of affective illness are being further pursued.

4. Peptides in CSF: Interrelationships with Neurotransmitter and Behavioral Alterations

a. Introduction: More than 20 neuropeptide substances have been suggested as putative CNS neurotransmitters or modulators. We have recently reviewed the literature indicating that essentially all of these substances have been tentatively identified and measured in the CSF of man. In many instances there is substantial evidence that CSF levels are regulated independently of those in the periphery and may be more closely associated with changes in brain. This provides one strategy for attempting to identify peptidergic alterations in neuropsychiatric disorders and to examine their postulated relationship to alterations in behavior, cognition, and affect. Neuropeptides have recently been reported to co-exist in the same neurons with classical neurotransmitter substances. Again, the CSF provides an opportunity for studying the potential interaction between both classical neurotransmitters and the recently discovered neuropeptides.

b. CSF Opiate-like Substances: In collaboration with Drs. D. Pickar, J.C. Ballenger, D. Naber, D.R. Rubinow, W.E. Bunney, Jr., and F.K. Goodwin, we have measured opiate-like substances in CSF utilizing a measure of both total CSF opiate binding activity and immunoreactive beta-endorphin. Total CSF opiate bind-

ing activity was not significantly different in depressed, manic, or improved patients compared to normal volunteers. CSF opiate binding activity from baseline and probenecid lumbar punctures (LPs) was correlated ($r = .73$, $p < .01$) in 16 patients, indicating the relative stability of this measure across two different LP procedures. Although there were no significant differences related to affective state, interesting relationships between CSF opiate activity and anxiety were observed. In depressed patients, those with higher nurse-rated anxiety showed significantly higher opiate binding activity ($r = .47$, $p < .01$, $n = 36$). These data are of particular interest in relation to the recent observation that a variety of stresses may be associated with the release not only of ACTH but also beta-endorphin in several experimental paradigms. The relationship between opiate activity in CSF and anxiety is also intriguing in relation to the differential findings in normal volunteers. Utilizing a different measure of anxiety, i.e., self-rated state anxiety at the time of the LP, it was observed that normal volunteers with higher CSF opiate binding activity showed significantly lower levels of subjective self-rated anxiety ($r = -.40$, $p < .05$, $n = 37$). These findings suggest that there may be complex interrelationships between opiate substances in CSF and different measures of acute and chronic anxiety in normal volunteers and depressed patients.

CSF beta-endorphin measured by radioimmunoassay was also not significantly different in unipolar and bipolar depressed patients compared to manic patients or normal volunteers. Preliminary evidence suggested that CSF immunoreactive beta-endorphin was differentially related to personality characteristics in female compared to male volunteers. Male volunteers showed a positive relationship between beta-endorphin and assaultiveness on the Buss-Durkee Rating Scale ($r = .77$, $p < .0002$) and on the Trait Hostility Scale ($r = .48$, $p < .04$), while female volunteers showed positive correlations between immunoreactive beta-endorphin and depression, social introversion, and negativity. These highly preliminary findings require replication, but are suggestive of the possibility that peptides measured in CSF might be associated with alterations in anxiety and certain personality variables, even though they are not different in diagnostic subgroups of patients with affective illness.

c. **Somatostatin in CSF:** CSF somatostatin has been measured in the CSF of affectively ill patients and normal volunteers by sensitive radioimmunoassay in collaboration with Drs. D.R. Rubinow, S. Reichlin and P.W. Gold. Dr. Rubinow found that CSF somatostatin was significantly decreased in depressed patients compared to those re-studied in the euthymic state or compared to normal volunteer controls. These findings replicate those of Gerner et al. of state-related decreases in CSF somatostatin in depression. CSF somatostatin in affectively ill patients was significantly and inversely correlated with the number of hours of sleep in the night prior to the LP. These data are consistent with those in the animal literature that somatostatin decreases a variety of sleep parameters including total sleep. As noted above, carbamazepine significantly decreased CSF somatostatin, while other psychotropic drugs produced no significant alterations and the relatively specific blocker of serotonin reuptake, zimelidine, significantly increased CSF somatostatin. These findings thus open new areas for exploration of the possible role of somatostatin decreases in depression, relative increases in relationship to degree of sleep disturbance, and in the possible mechanism of action of carbamazepine which has an interesting spectrum of clinical efficacy in affective illness, seizure disorders, and paroxysmal pain syndromes.

d. CRH, ACTH, and Cortisol: Drs. D.R. Rubinow, P.W. Gold, and J.C. Ballenger have extensively studied pituitary-adrenal dysregulation in affective illness. They have observed significantly higher excretion of urinary free cortisol in unipolar and bipolar depressives compared to normal volunteers, with significantly lower levels in manic patients. These findings are paralleled by a large literature of well-documented and replicated studies indicating that approximately 50% of depressed patients show evidence of cortisol hypersecretion measured either by escape from dexamethasone suppression, increased urinary free cortisol, or altered diurnal variation of cortisol secretion. In addition, Dr. Rubinow has documented marked state-related alterations in urinary free cortisol secretion and highly significant correlations in 8 AM plasma cortisol with severity of depression in cycling manic-depressive patients studied longitudinally. In our studies of urinary free cortisol and in the literature on dexamethasone suppression, severity of depression has not been well correlated with evidence of pituitary-adrenal axis disinhibition. It was particularly noteworthy to find that patients with higher levels of urinary free cortisol showed greater cognitive impairment on the Halstead Categories test of abstracting ability ($r = .48, p < .01$). These findings suggest that patients with higher levels of urinary free cortisol are more cognitively impaired, which is of interest in relationship to the high density of glucocorticoid binding sites measured in limbic structures, such as the hippocampus, which are thought to be critically involved in some aspects of learning and memory function. It is possible that either the high levels of cortisol or the neurochemical alterations underlying this abnormality are associated with this objective measure of cognitive impairment. These data are of some theoretical relevance, as well as of possible clinical significance, since depressed patients often have marked complaints of subjective decreases in cognitive and memory capacity. Dr. Gold has initiated studies of CRH infusions in affectively ill patients and controls as described in Project # Z01 MH 00452-08 BP.

e. Vasopressin and Oxytocin: Vasopressin and oxytocin are of considerable interest since a large body of experimental data in animals and preliminary studies in man suggest that they may have effects on learning and memory. Vasopressin has been measured in plasma and CSF in collaboration with Drs. P.W. Gold, D.R. Rubinow, and G. Robertson. Dr. Gold has observed that CSF values in non-psychotic bipolar depressed patients were significantly lower than those in the manic phase of the illness. In contrast, CSF oxytocin, measured in collaboration with Dr. D. Fisher, was found by Dr. Gold to be significantly decreased in manic patients compared to normal volunteers. The possible relationships of these findings to the syndromal and symptomatic alterations in mania and depression remain to be further explored but appear to be of considerable interest in their own right, as well as in relation to their serving as possible markers of hypothalamic dysfunction. Preliminary evidence suggests that vasopressin may be secreted directly into CSF independently of alterations in its peripheral levels. These and related data suggest that study of peptides in CSF may provide useful indirect markers of CNS peptide function and provide a basis for studying alterations in relationship to a variety of neuropsychiatric symptoms and syndromes. Drs. Rubinow, Gold, and Weingartner are studying the effects of infused oxytocin and vasopressin on mood and cognitive capacities of affectively ill patients and normal volunteers. Initial data suggest that vasopressin enhances, while oxytocin impairs, certain aspects of cognition and that vasopressin increases cortisol secretion.

A vasopressin binding site on human platelets has been tentatively identified in studies in collaboration with Dr. Berrettini. Based on the potency of various compounds tested, preliminary data suggests that this binding site may have characteristics similar to that of the renal vasopressin receptor. These studies raise the possibility that one may be able to indirectly measure vasopressin receptor function in man in addition to other measures such as that of vasopressin itself in CSF.

5. Life Charting the Course of Affective Illness

In collaboration with Dr. P. Roy-Byrne, K. Squillace, T. Porcu, and D. Davis, we have recently completed the first phase of analysis of the life course of illness in 66 unipolar and bipolar patients, and data are now available on 96 such patients. In addition to this detailed retrospective life chart evaluation, cyclicity within NIMH has been precisely characterized. Differential characteristics of unipolar patients have been noted. Compared to bipolars, unipolar patients have significantly greater numbers of weeks hospitalized per years ill, but decreased number of total depressive episodes or episodes in the year prior to NIMH admission, while they experienced more weeks ill in the year prior to NIMH admission. Thus, their illness was characterized by decreased cyclicity but equal or greater impairment in functioning. These differences persisted during hospitalization at NIMH, with unipolar patients showing decreased number of depressive episodes compared with bipolar patients.

Female compared to male patients showed an increased proclivity for rapid cycling and significantly greater number of episodes in the year prior to NIMH admission (5.9 ± 1.1 , $n = 34$ compared to 1.7 ± 0.2 , $n = 29$). Females were overly represented in the group of rapid cycling patients. Male and female rapid cycling patients ($n = 20$) compared to slow cycling patients ($n = 29$) had significantly longer duration(s) of illness, more hospitalizations for depression, greater numbers of weeks hospitalized, as well as greatly increased total lifetime episodes of affective illness (56.7 ± 18.6 compared to 8.2 ± 1.2). The rapidity of cycling, defined as four or more episodes in the year prior to NIMH hospitalization, continued to be an excellent predictor of the course of illness at NIMH with rapid cyclers showing significantly more manic, depressive, and total episodes at NIMH. In the total group of 47 bipolar patients, the number of episodes in the year prior to NIMH admission was highly correlated with the number of episodes observed during NIMH hospitalization ($r = .69$, $p < .0001$). Thus, the prior course of cycling appears to be the best predictor of subsequent course of illness. Numbers of episodes of mania and of depression prior to NIMH hospitalization were highly correlated within 32 patients studied ($r = .90$, $p < .0001$). This same symmetry between number of observed manic and depressive episodes was again documented during the NIMH hospitalization where number of manic episodes correlated with number of depressive episodes ($r = .91$, $p < .001$) in 49 bipolar patients studied.

The majority of affectively ill patients were observed to show a progressive increase in rapidity of cycling as a function of episode number, as observed earlier by Kraepelin and more recently by Goodwin, Zis, Grof and associates. Thus, the study of the longitudinal course of affective illness provides a template not only for assessing the phenomenology of the illness and its response to treatment interventions with agents such as lithium and carbamazepine, but also refocuses on possible biological mechanisms underlying the recurrent and, at times, progressive aspects of affective illness. For example, we have noted

above findings of increased noradrenergic function in depression associated with rapidity of cycling. Studies in laboratory animals of behavioral sensitization to stimulants and stressors and of electrophysiological kindling may provide insights into different types of mechanisms underlying the progressive evolution of behavioral disturbances in response to repetition of the same stimulation over time.

We suggest that the life charting process is a useful clinical as well as research tool and may help focus on possible environmental precipitants and dynamically significant events and stresses that may be temporally related to affective episodes. It also allows precise characterization of the degree of longitudinal response to newly available pharmacological agents. Recent data of Wehr and Goodwin have emphasized that some pharmacological interventions such as the tri-cyclic antidepressants may actually result in increased rapidity of cycling. The life chart methodology provides a useful instrument for following this problematic side effect. We have also observed in several patients that lithium carbonate may paradoxically increase the rate of rapid cycling in addition to significantly decreasing the duration of recurrent depressive episodes. The particular vulnerability of female patients to experience extremely rapidly cycling manic-depressive illness would appear a fruitful area for further study. It also helps to focus on possible endocrine concomitants of this process.

6. Menstrually-Related Mood Dysfunction

A relationship between mood and behavior and menstrual function has been described with respect to a number of disorders including premenstrual tension, post-partum depression, epilepsy (so-called catamenial epilepsy), and menopausal dysphoria. Dr. D.R. Rubinow has initiated a series of studies to investigate the relationship between mood disorders and the menstrual cycle. These studies include: development of a questionnaire which is being employed to help determine the incidence and nature of affective symptoms in relation to the menstrual cycle; assessment of the precision of the relationship between mood changes and the menstrual cycle utilizing daily self-ratings and daily temperature recordings; investigation of hormonal activity employing periodic blood samples and neuroendocrine tests; and assessment of the efficacy of progesterone, a synthetic progestin, and carbamazepine in the treatment of established menstrually-related mood syndromes. The results of such a study may: 1) determine whether a specific association between depressive symptoms and menstrually-related phenomena (menstruation, post-partum depression, menopause, hormone-induced behavioral change) can be established; 2) reveal the incidence of the entrainment of depressive symptoms to the menstrual cycle; 3) help elucidate the nature of the "switch" mechanism in affective disorders and periodic psychosis; and 4) determine the efficacy of pharmacologic agents believed useful in the treatment of menstrually-related mood disorders.

At this point, questionnaires have been filled out by 500 women and 20 women are entering the hormonal assessment phase of the study, having completed the baseline evaluation phase.

7. Depressive Subtypes and Symptoms in Relation to Regional Localization of Function

a. Atypicality of Depression: In collaboration with Dr. E.K. Silberman, we have devised an atypicality of depression rating scale in order to

more precisely characterize the range of atypical depressive presentations in patients who otherwise meet formal Research Diagnostic Criteria for primary affective illness. Older patients and those with bipolar I affective illness had more typical presentations than those of unipolar or bipolar II patients. The more typical patients showed increased rapid cycling in the year prior to NIMH admission although, interestingly, decreased numbers of total hospitalizations compared to the atypical patients. Atypical depressed patients also showed more variance in biological measures such as those of the noradrenergic system, further suggesting that the range of clinical presentations may be related to the range of biological variables that have been hypothetically linked to depressive illness. It was of interest that both typical and atypical depressed patients showed similar degrees of cortisol hypersecretion.

b. Psychosensory Phenomena: In collaboration with Drs. E.K. Silberman and J-P. Boulenger we have developed an interview rating scale designed to measure signs and symptoms that are usually associated with psychomotor epilepsy (complex partial seizures). We have studied these phenomena in patients with primary affective illness without evidence of seizure disorders, in patients with documented evidence of temporal lobe epilepsy, and in a medical control group of hypertensive patients. Compared to the control group, patients with both affective illness and epilepsy showed a highly significant increased incidence in the number of these signs and symptoms. To the extent that psychosensory distortions and related symptoms usually associated with temporal lobe epilepsy are occurring with a high incidence in patients with primary affective illness, it might suggest that the neural substrates involved in complex partial seizures overlap with affective illness. Patients with greater numbers of psychosensory symptoms responded better to lithium carbonate, and preliminary data suggest that this is not the case for carbamazepine, as we would have predicted.

c. Psychological, Structural, Metabolic, and Electrophysiological Approaches to Regional Brain Function in Affective Illness: A variety of psychological test batteries are employed to assess possible alterations in regional brain function in patients with affective illness including the Luria Battery, the Halstead Category Test, tachistoscopic presentation to assess hemispherical laterality, and other cognitive tests studied in collaboration with Dr. E.K. Silberman. Consistent with patients' subjective sense of cognitive impairment during depression, marked impairment in cognitive function has been documented on the Halstead Category Test.

Degree of cognitive dysfunction correlated with increases in urinary free cortisol secretion, suggesting that cortisol hypersecretion may be primarily or secondarily related to this important subjective and objective deficit in depressed patients. The Luria Battery provides another approach to assessment of possible regional areas of dysfunction and has been completed in more than 45 patients.

Computerized axial tomography (CAT) scans have been performed on our patients with affective illness and reveal a similar range of increased ventricular brain ratios (VBRs) comparable to those observed in schizophrenic patients. We are currently assessing the clinical and biological concomitants of this evidence of altered brain structure in a subgroup of affectively ill patients (in collaboration with Drs. C.H. Kellner and W.H. Berrettini). Dr. Kellner observed that

patients with the greatest urinary free cortisol excretion had the largest VBRs ($r = .81, p < .02$). The initial findings in 10 subjects have been extended to include 25 patients. These data are consistent with those in the literature indicating that treatment with ACTH or exogenous glucocorticoids such as dexamethasone is associated with reversible atrophy and enlarged ventricles on CAT scan. This literature and our findings suggest that "structural" alterations in brain on the CAT scan may not be as irreversible as previously thought and that exogenous and perhaps endogenous biochemical changes may be important mediators of this brain measure which is receiving increasing attention as a possible concomitant of some patients with schizophrenic illness.

As described in detail elsewhere, topographic mapping of EEG frequencies and averaged evoked response is being conducted in collaboration with Drs. R. Cohen, L. DiLisi, H. Holcomb and M.S. Buchsbaum. These studies, in conjunction with positron emission tomography (PET) scan, may provide important evidence of electrophysiological and/or metabolic regional dysfunction in affective illness. These findings can then be compared with ongoing psychological, longitudinal, physiological, and biochemical assessment of affectively ill patients in order to complete a coherent and comprehensive assessment of possible interrelationships of these measures in affective illness. Initial studies indicate that acutely ill and improved affective disorder patients show a nonspecific pattern of hypofrontality similar to that observed in schizophrenia and other patient populations. These and other data indicate that relative hypofrontality in glucose utilization is not specific to the psychopathology of schizophrenia and other alterations more intimately related to affective symptomatology remain to be elucidated.

8. Laboratory Studies of Behavioral Sensitization and Electrophysiological Kindling

a. Stimulant-induced Behavioral Sensitization: A series of studies have been designed to investigate the mechanisms underlying increased behavioral responsivity to the same dose of a psychomotor stimulant such as cocaine. Animals administered cocaine (10 mg/kg i.p.) once-daily showed increasing amounts of locomotor hyperactivity and stereotypy to the same dose over time. An environmental context and conditioning component has been demonstrated. Animals repeatedly treated with cocaine in the context of the test cage showed greater degrees of hyperactivity and stereotypy than animals receiving identical doses in a different environment. Significant differences also existed when animals were challenged with a saline injection, again suggesting a conditional component to cocaine-induced behavioral sensitization. Brattleboro homozygote rats lacking vasopressin showed deficient onset, maintenance, and persistence of cocaine-induced behavioral sensitization compared to their heterozygote litter-mate controls. Another group of Brattleboro homozygotes were able to show similar degrees of acute reactivity to high doses of cocaine, indicating that the homozygotes were not just unable to show similar degrees of motor activation. We have replicated the original findings showing that vasopressin replacement will reverse the deficit in cocaine-induced behavioral sensitization.

In addition, female compared to male rats are more responsive to the same dose of cocaine. They demonstrate similar behavioral sensitization to repeated injections of cocaine at approximately half the dose (5 mg/kg) of that used in males (10 mg/kg, i.p.).

Studies in collaboration with Dr. K. Zander have assessed cross-sensitization between cocaine-induced hyperactivity and several types of stresses such as those induced by tail pinch. It appears that type of stress, its intensity, and longitudinal time course are important determinants of whether animals will show increased or decreased responsivity to a cocaine challenge. Some aspects of the response to repeated stress showed clear-cut sensitization effects, while others appeared to show adaptation or tolerance. A 40 kHz vocalization showed increasing amplitude of response to repetition of the same level of tail pinch stress over time.

b. Electrophysiological Kindling: Repeated, intermittent electrical stimulation of the brain results in increasing duration, spread, and complexity of electrical after-discharges culminating in the appearance of major motor seizures to a previously subthreshold stimulation. We have employed this procedure, as described by Goddard et al., in order to study long-lasting changes in neural and behavioral excitability that accompany this process. Following electrical kindling of the amygdala, rats showed decreased spontaneous and cocaine-induced exploratory activity, while they showed increased convulsive susceptibility to a related local anesthetic, lidocaine. Repeated injections of the same dose of lidocaine (65 mg/kg, i.p.) lead to an increasing incidence, severity, and duration of seizures to the same dose over time. This effect does not appear to be a pharmacokinetic one, as blood levels of lidocaine and its metabolite are not increased with chronic administration. Moreover, if lidocaine-induced excitability and seizures are blocked by the co-administration of diazepam, no seizure sensitization occurs. Finally, repeated lidocaine-induced seizures sensitize to electrophysiological kindling of the amygdala such that amygdala-kindling proceeds three times faster following lidocaine pretreatment compared to saline controls. These data suggest some degree of cross-sensitization between electrical and chemical modes of kindling.

Behavioral alterations persist in the interictal period following lidocaine-induced seizures. In collaboration with Drs. L. Sokoloff, C. Kennedy and their associates in the Lab. of Neurochemistry, it has been demonstrated that lidocaine-induced seizures relatively selectively increase metabolic activity in limbic system structures, particularly amygdala, hippocampus, perirhinal, and cingulate cortical areas. It is, thus, of interest that increases in irritability and resistance to capture are prominent following lidocaine seizures but not following seizures induced by electroconvulsive shock or pentylenetetrazol (Metrazol). The changes in irritable and aggressive behavior following lidocaine seizures persist for some days into the interictal period. This paradigm would therefore appear to be a useful one in exploring the relationship of seizures with some specificity for limbic structures to alterations in aggressive behavior.

Studies with S. Weiss have shown that carbamazepine (15 mg/kg, i.p.) is a potent inhibitor of completed amygdala-kindled seizures, but at this dose is not effective in suppressing the development of kindling in the rat. As noted above, we have recently observed that carbamazepine-10,11-epoxide is more highly correlated with the degree of psychotropic response in our patients than is the parent compound. We have demonstrated that the metabolite carbamazepine-10,11-epoxide is also effective in inhibiting amygdala-kindled seizures, although it is slightly less potent than carbamazepine itself. Possible mechanisms underlying the kindling process itself are being studied in collaboration with J. Patel and P. Marangos.

Preliminary evidence has been obtained that 24 hours following kindling, there is selective phosphorylation of a 45K protein in the amygdala bilaterally; it is not observed following repeated ECT seizures. The identity of this protein, the duration of its charge, and relevance to the physiology of kindling are being explored.

Preliminary evidence, in collaboration with P. Marangos and J. Patel, suggests that adenosine receptors are not significantly affected by kindling, but that calcium channels marked by ^3H -nitrendipine may be altered by both electroconvulsive kindling of the amygdala and by lidocaine kindling.

c. Electroconvulsive Shock Inhibits Amygdala Kindling: The clinical utility of an anticonvulsant such as carbamazepine appears paradoxical in relation to electroconvulsive therapy or the induction of major motor seizures also having therapeutic efficacy in both manic and depressed phases of affective illness. One possible explanation of this paradox emerges from two separate studies in collaboration with Dr. F.W. Putnam and N. Contel demonstrating that the major motor seizures of electroconvulsive shock (ECS) are themselves anticonvulsant to amygdala-kindled seizures. Pretreatment with ECS six hours prior to amygdala kindling markedly inhibits development of kindled seizures compared to sham ECS or compared to ECS administered immediately after kindling. In a second study, we used a more clinically relevant paradigm. Animals were kindled to their first stage for major motor seizure and then were treated with single or seven daily ECS or sham ECS. Following this seven-day interval, amygdala kindling was resumed. Chronic ECS, but not one ECS followed by a seven-day delay, markedly inhibited amygdala-kindled seizures for up to five days compared to sham ECS controls. Taken together these two studies indicate that the major motor seizures of ECS can, in two different time frames, exert marked anticonvulsant effects on amygdala-kindled seizures. These data raise the possibility that the efficacy of electroconvulsive therapy in patients with affective illness could be related to effects mediating its anticonvulsant actions.

D. Proposed Course of Project

As carbamazepine is emerging as an effective treatment modality in some patients with manic-depressive and schizoaffective illness, we will attempt to further delineate clinical and biological markers of carbamazepine response. Preliminary evidence suggests that many patients who clearly do not respond to lithium carbonate will respond to carbamazepine. It will be increasingly important to establish whether response to carbamazepine compared to lithium carbonate delineates separate subgroups of patients with affective illness. It is also possible that carbamazepine may be more effective in later stages of the illness, particularly when the patients are in a treatment-resistant, rapid-cycling phase of illness. Preliminary evidence indicates that patients with a family history of psychiatric illness do not differ in their response to carbamazepine as has been reported for lithium and postulated in the opposite direction for carbamazepine. Genetic variables will be further examined in relationship to carbamazepine response. Mild EEG abnormalities, cognitive alterations, or alterations in psychosensory function also do not appear to predict response to carbamazepine. The degree of generalization of carbamazepine response to other anticonvulsant agents such as phenytoin or valproic acid will be another area of both clinical and theoretical import. This is also particularly the case in light of our recent finding

that electroconvulsive therapy may be exerting potent anticonvulsant effects on limbic system seizures. Are anticonvulsant effects of a variety of treatment modalities linked to therapeutic response in affective illness? Carbamazepine is clearly useful in pain syndromes that do not involve a convulsive process, and effectiveness of anticonvulsant agents in a subgroup of patients with affective illness does not imply an underlying ictal process. The possible mechanisms of action of carbamazepine studied in our clinical population, as well as in behavioral pharmacological models and at more basic molecular levels, will also be pursued.

Topographic mapping of electroencephalographic activity and PET scan techniques will be explored in collaboration with Drs. R. Cohen, H. Holcomb, L. DeLisi and M.S. Buchsbaum, not only in affectively ill patients compared to controls, but also as they might predict or correlate with treatment response. Further clinical and laboratory work will be pursued to investigate whether carbamazepine's anticonvulsant metabolite carbamazepine-10,11-epoxide has active psychotropic properties.

The interrelationship of classical neurotransmitter substances with the putative CNS neurotransmitter peptides will be explored in both patients with affective illness and anxiety disorders in collaboration with Drs. D.C. Jimerson and T.W. Uhde. A variety of techniques are in place for measurement of neurotransmitter and receptor function in both classical neurotransmitter systems and in the peptide systems in man. These will be correlated with behavioral alterations and changes in mood and cognitive functioning in patients with mood and anxiety disorders.

As described in detail in Project #Z01 MH 00071-03 BP, Dr. T.W. Uhde will continue to explore the similarities and differences in patients with panic anxiety syndromes and those with affective illness in terms of acute symptomatology, longitudinal course of illness, and response to pharmacological agents. Catecholamines appear to be altered in both the mood disorders and in panic anxiety disorders. Response to treatments which act on catecholamine systems such as clonidine will be compared and contrasted in both patient populations. The clinical utility of carbamazepine will also be explored in this syndrome. Dr. F.W. Putnam will complete some phases of his studies of psychological, psychophysiological, and neural mechanisms underlying patients with multiple personality syndrome and initiate others. An extensive questionnaire for 100 patients has been completed which better delineates symptoms and course of illness of multiple personality syndrome, and further supports the important etiological role of childhood physical and/or sexual trauma (see Project # Z01 MH 00072-03 BP).

Dr. D.R. Rubinow is also continuing to develop a new combined inpatient and outpatient focus on patients with menstrually-related exacerbation of mood and behavior disorders. He will be examining this problem from a clinical and endocrinological point of view, and as a model for studying the acute onset and offset of affective dysfunction. Similar studies will be pursued utilizing sleep deprivation, which represents another non-pharmacological means of inducing rapid and non-pharmacologically related improvement in mood, as well as examining mechanisms that may underlie exacerbation of depression that occurs regularly when patients return to sleep.

Work in animal models will continue to focus on possible mechanisms underlying behavioral sensitization and electrophysiological kindling. In collaboration with P. Marangos and J. Patel, neurotransmitter receptors, protein phosphorylation, and ion channels will be examined as possible mediators or modulators of the electrophysiological kindling paradigm. Studies of behavioral and biochemical response to repeated stress will be performed in collaboration with S. Weiss and A. Pert. The role of environmental context and conditioning will also be examined in these paradigms.

E. Significance to Biomedical Research and the Program of the Institute

Findings in several research areas are of considerable clinical and theoretical significance. Carbamazepine is emerging as a new treatment for manic-depressive illness; it is effective in some patients who do not respond to lithium carbonate. In addition, clinical and basic work exploring the mechanism of action of this compound alone or in comparison to lithium carbonate and other clinically effective psychotropic agents may provide new leads to the understanding of mechanisms of action of effective anti-manic and antidepressant drugs and mechanisms underlying affective dysregulation. Study of endocrine and peptide substances in man and animals may also provide new conceptual and practical approaches to the relationship between manic and depressive symptoms and biochemistry. Examination of the interaction between classical neurotransmitters and the peptides should prove fruitful in understanding normal and pathological functioning. The multidisciplinary assessment of our patients' mood, behavior, cognition, physiology, and biochemistry will allow more precise characterization of important biobehavioral relationships. Study of the mechanisms underlying behavioral sensitization and kindling should yield important information regarding the coding of long-term changes in the CNS. Thus, basic and clinical research have led to important developments in neurobiology and the development of a new treatment for affective illness with carbamazepine. Drs. Uhde and Post received the Biological Psychiatry Society and American Psychiatric Association research prizes, respectively, for their clinical research studies.

Publications:

Post, R.M., Ballenger, J.C., Uhde, T.W., Smith, C., Rubinow, D.R., and Bunney, W.E., Jr.: Effect of carbamazepine on cyclic nucleotides in CSF of patients with affective illness. Biol. Psychiatry 17: 1037-1045, 1982.

Hare, T.A., Wood, J.H., Manyam, B.V., Gerner, R.H., Ballenger, J.C., and Post, R.M.: Central nervous system gamma-aminobutyric acid activity in man related to age and sex as reflected in cerebrospinal fluid. Arch. Neurol. 39: 247-249, 1982.

Silberman, E.K. and Post, R.M.: Atypicality in primary depressive illness: a preliminary survey. Biol. Psychiatry 17: 285-304, 1982.

Post, R.M. and Uhde, T.W.: Biological relationships between melancholic depression and mania. L'Encephale VIII: 213-228, 1982.

Post, R.M., Uhde, T.W., Putnam, F.W., Ballenger, J.C., and Berrettini, W.H.: Kindling and carbamazepine in affective illness. J. Nerv. Ment. Dis. 170: 717-731, 1982.

Post, R.M., Contel, N.R., and Gold, P.W.: Impaired behavioral sensitization to cocaine in vasopressin deficient rats. Life Sci. 31: 2745-2950, 1982.

Post, R.M.: Use of the anticonvulsant carbamazepine in primary and secondary affective illness: clinical and theoretical implications. Psychol. Med. 12: 701-704, 1982.

Zuckerman, M., Ballenger, J.C., Jimerson, D.C., Murphy, D.L., and Post, R.M.: A correlation test in humans of the biological models of sensation seeking, impulsivity, and anxiety. In Zuckerman, M. (Ed.): Biological Bases of Sensation Seeking, Impulsivity, and Anxiety. New Jersey, Lawrence Erlbaum Associates, 1983, pp. 229-248.

Post, R.M. and Contel, N.R.: Human and animal studies of cocaine: implications for development of behavioral pathology. In Creese, I. (Ed.): Stimulants: Neurochemical, Behavioral, and Clinical Perspective. New York, Raven Press, 1983, pp. 169-203.

Post, R.M.: Behavioral effects of kindling. In Parsonage, M. (Ed.): Advances in Epileptology: XIVth Epilepsy International Symposium. New York, Raven Press, 1983, pp. 173-180.

Boulenger, J.-P., Patel, J., Post, R.M., Parma, A.M., and Marangos, P.J.: Chronic caffeine consumption increases the number of brain adenosine and benzodiazepine receptors. Life Sci. 32: 1135-1142, 1983.

Del Zompo, M., Post, R.M., and Tallman, J.F.: Properties of two benzodiazepine binding sites in spinal cord. Neuropharmacology 22: 115-118, 1983.

Post, R.M.: Stereotypy, spikes, schizophrenia, and seizures. Editorial II. Biol. Psychiatry 18: 410-413, 1983.

Post, R.M., Gold, P.W., Rubinow, D.R., Bunney, W.E., Jr., Ballenger, J.C., and Goodwin, F.K.: Cerebrospinal fluid as a neuroregulatory pathway: CSF peptides in neuropsychiatric illness. In Wood, J.H. (Ed.): The Neurobiology of Cerebrospinal Fluid, Vol. II. New York, Plenum Press, in press.

Ballenger, J.C., Post, R.M., and Goodwin, F.K.: The neurochemistry of cerebrospinal fluid in normal subjects: relationship between biological and psychological variables. In Wood, J.H. (Ed.): The Neurobiology of Cerebrospinal Fluid, Vol. II. New York, Plenum Press, in press.

Major, L.F., Lerner, P., Dendel, P.F., and Post, R.M.: Dopamine-beta-hydroxylase in cerebrospinal fluid: a possible indicator of central noradrenergic activity. In Wood, J.H. (Ed.): The Neurobiology of Cerebrospinal Fluid, Vol. II. New York, Plenum Press, in press.

Post, R.M., Rubinow, D.R., and Ballenger, J.C.: Conditioning, sensitization, and kindling: implications for the course of affective illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Post, R.M., Ballenger, J.C., Uhde, T.W., and Bunney, W.E., Jr.: Efficacy of carbamazepine in manic-depressive illness: implications for underlying mechanisms. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Rubinow, D.R., Post, R.M., Gold, P.W., Ballenger, J.C., and Wolff, E.A.: The relationship between cortisol and clinical phenomenology of affective illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Post, R.M., Pickar, D., Ballenger, J.C., Naber, D., and Rubinow, D.R.: Endogenous opiates in CSF: relationship to mood and anxiety. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Ballenger, J.C. and Post, R.M.: Neurobiological concomitants of depression and anxiety in normal individuals. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Squillace, K.M., Post, R.M., Savard, R., and Erwin, M.: Life charting of the longitudinal course of affective illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Carman, J., Wyatt, E., Smith, W., Post, R.M., and Ballenger, J.C.: Calcium and calcitonin in bipolar affective disorder. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

- Post, R.M., Jimerson, D.C., Ballenger, J.C., Lake, C.R., Lerner, P., Uhde, T.W., and Goodwin, F.K.: CSF norepinephrine and its metabolites in manic-depressive illness. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.
- Jimerson, D.C. and Post, R.M.: Psychomotor stimulants and dopamine agonists in depression. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.
- Roy-Byrne, P.P., Uhde, T.W., and Post, R.M.: Sleep deprivation: clinical and theoretical implications. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.
- Post, R.M., Uhde, T.W., and Ballenger, J.C.: The efficacy of carbamazepine in affective illness. In Usdin E. (Ed.): Frontiers in Biochemical and Pharmacological Research in Depression. New York, Raven Press, in press.
- Post, R.M., Uhde, T.W., Ballenger, J.C., Chatterji, D.C., Greene, R.F., and Bunney, W.E., Jr.: CSF carbamazepine and its -10,11-epoxide metabolite in manic-depressive patients: relationship to clinical response. Arch. Gen. Psychiatry, in press.
- Post, R.M.: Biochemical and physiological mechanisms of action of carbamazepine in affective illness. In Usdin, E. (Ed.): Frontiers in Neuropsychiatric Research. London, MacMillan Press, Ltd., in press.
- Post, R.M., Uhde, T.W., Rubinow, D.R., Ballenger, J.C., and Gold, P.W.: Biochemical effects of carbamazepine: relationship to its mechanisms of action in affective illness. Prog. Neuro-Psychopharmacol. & Biol. Psychiat., in press.
- Post, R.M., Uhde, T.W., Ballenger, J.C., and Squillace, K.M.: Prophylactic efficacy of carbamazepine in manic-depressive illness. Am. J. Psychiatry, in press.
- Post, R.M.: Introductory Comments for Symposium #12: CSF concentrations of amines and metabolites: clinical and methodological perspectives. In Usdin, E. (Ed.): Catecholamines: Neurology and Neurobiology. New York, A.R. Liss, Inc., in press.
- Post, R.M. and Uhde, T.W.: Psychotropic effects of carbamazepine and its mechanism of action in affective illness. In Burrows, G.D. and Werry, J.S. (Eds.): Advances in Human Psychopharmacology, Vol. IV. Greenwich, Connecticut, JAI Press, Inc., in press.
- Post, R.M., Pitem, I., and Contel, N.R.: Lidocaine-induced seizures produce cross sensitization to electrical kindling of the amygdala. Exp. Neurol., in press.

Post, R.M. and Uhde, T.W.: Carbamazepine in the treatment of mood and anxiety disorders: implications for limbic system mechanisms. In Proceedings of the Meeting of the British Association for Psychopharmacology and Association Francaise de Psychiatrie Biologique, Paris, France, October, 1982. London, John Wiley and Sons, in press.

Post, R.M. and Uhde, T.W.: Alternatives to lithium: a focus on carbamazepine. In Ayd, F.J. (Ed.): Affective Disorders Reassessed: 1983. Baltimore, Ayd Medical Publications, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00071-03 BP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiological Correlates and Treatment of Panic and Related Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) T.W. Uhde, M.D., Chief, Unit on Anxiety and Affective Disorders, BPB, NIMH		
COOPERATING UNITS (if any) 3 -West Nursing Dept., NIH; CPB, LPP, NS, LCS, NIMH; VA Medical Ctr., Bronx, New York; University of California at Irvine; University of Oregon; San Diego Veteran's Medical Center		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5	PROFESSIONAL: 4	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Patients with pathological degrees of anxiety who meet DSM III criteria for generalized anxiety, <u>panic</u> or <u>phobic disorders</u> are evaluated using psychological, physiological, and biochemical methodologies. Patients with Major Affective Illness, particularly those with a significant anxiety component, are also eligible for participation in the program. Particular attention is given to the role of the <u>noradrenergic neurotransmitter system</u> as assessed by: 1) measurement of the metabolite MHPG in urine, plasma, and CSF; 2) adrenergic receptor number and function in platelets; and 3) neuroendocrine and behavioral response to the alpha-2 adrenergic agonist <u>clonidine</u>, and antagonist <u>yohimbine</u>. Research investigating the relationship of noradrenergic function to other neurotransmitter systems such as those which influence opiate, adenosine, and GABA-benzodiazepine function also have been initiated. Other approaches to understanding the pathophysiology of anxiety and its potential treatment with <u>alprazolam</u>, <u>carbamazepine</u>, <u>clonidine</u>, <u>imipramine</u>, and <u>propranolol</u> will be explored. </p>		

Collaborators:

R.M. Post, M.D.	Chief, Biological Psychiatry Branch, NIMH
J.P. Boulenger, M.D.	Visiting Associate, BPB, NIMH
B. Scupi, M.S.W.	Clinical Social Worker, BPB, NIMH
M. Geraci	Psychiatric Nurse, 3-West Nursing Dept., NIH
M. Buchsbaum, M.D.	Professor of Psychiatry, Univ. of California at Irvine
J.C. Gillin, M.D.	Professor of Psychiatry, San Diego Veteran's Medical Ctr.
W. Mendelson	Chief, Unit on Sleep Studies, CPB
T.P. Zahn, Ph.D.	Research Psychologist, LPP, NIMH
R. Watson, M.D.	Medical Staff Fellow, NHLB
M. Kafka, Ph.D.	Research Physiologist, NS, NIMH
L. Siever, M.D.	Associate Professor, VA Medical Center, Bronx, New York
D.C. Jimerson, M.D.	Staff Psychiatrist, LCS, NIMH
A. Lewy, M.D.	Staff Psychiatrist, University of Oregon

I. Project Description

A. Objectives

This project employs a multidisciplinary team in the study and treatment of pathological anxiety, major affective and related mood disorders.

B. Methods Employed

1. Subjects

a. Patients who meet Research Diagnostic Criteria for panic, phobic, and generalized anxiety disorders as well as patients who meet DSM III criteria for major affective illness are candidates for participation in the project. Patients are studied and treated on the 3-West Clinical Research Unit, or through the Unit on Anxiety and Affective Disorder's Outpatient Division. A number of previously validated scales to measure state and trait anxiety are utilized and an analogue anxiety scale and panic anxiety scale have been developed to more adequately assess the relationship among state anxiety, phobic anxiety, avoidance behavior, and depressive symptomatology.

b. Normal volunteers are also accepted into the project to provide control data as well as to assess the relationship between normal state anxiety and selected psychological and biological variables.

2. Psychological and Biological Evaluation

a. Baseline Evaluation. During an initial evaluative period patients undergo extensive neurological, psychological, biochemical, and neurophysiological evaluation. This initial evaluation is indicated due to the heterogeneous nature of the panic and phobic disorders. Anecdotal reports suggest that many medical illnesses may present as or exacerbate pre-existing conditions of pathological anxiety. However, no research has systematically studied a large number of panic and phobic patients to determine the incidence and prevalence of these associated disorders.

b. Life Chart Methodology. A life chart technique has been developed in collaboration with Dr. J.-P. Boulenger, Dr. B. J. Vittone, M. Geraci, and B. Scupi to plot the frequency, intensity, and interval between panic attacks and agoraphobia, so that the development, recurrence, and progression of the panic and phobic disorder can be assessed. This is an important aspect of the project because few systematic studies have been conducted on the natural progression of these disorders.

c. Caffeine and Anxiety. In collaboration with Dr. J.-P. Boulenger, a caffeine inventory has been developed to assess the effects of caffeine on anxiety and related symptoms in panic anxious and affectively ill patients and normal volunteers. In addition caffeine is administered in an oral challenge to assess clinical, physiological, and neuroendocrine responses to this widely consumed drug which is also known to influence noradrenergic, benzodiazepine, and adenosine function.

d. Sleep Research. Electroencephalographic sleep recordings are obtained for three consecutive nights. Although many panic anxious patients, like endogenously depressed individuals, have improved sleep following treatment with tricyclic and monoamine oxidase inhibitors, nothing is known about the sleep architecture of panic and phobic anxious patients. In collaboration with Drs. J.C. Gillin and Dr. W. Mendelson, this ongoing research represents the first attempt to our knowledge to evaluate the sleep profile of this patient population.

e. Insensitivity Index. Using threshold and signal detection methodology, an index of pain insensitivity is obtained in patients and normal volunteers following the intravenous administration of clonidine 2 µg/kg and placebo.

f. Galvanic Skin Response. The effects of alprazolam and imipramine and selected standard anxiolytics on physiological measures of galvanic skin response, reaction time to auditory tones, pulse, and respiratory rate are studied in panic and phobic anxious patients and age-matched normal volunteers. This investigation is performed in collaboration with Dr. T. Zahn.

g. Echocardiography. Echocardiography is obtained in patients and age-matched controls to assess the presence or absence of mitral valve prolapse. These data are obtained in collaboration with Dr. R. Watson who is blind to the diagnosis of each patient or normal volunteer when echocardiography and auscultation are performed.

h. Clonidine -- An Alpha-Adrenergic Agonist. Clonidine is administered intravenously to anxious and affectively ill patients and volunteers to assess clinical, physiological, and neuroendocrine responses to this noradrenergic drug.

i. Yohimbine -- An Alpha-Adrenergic Antagonist. Yohimbine is administered in an oral challenge to panic anxious and affectively ill patients and normal controls to assess the clinical and biochemical effects of this noradrenergic antagonist which is known to potently increase noradrenergic function in the animal.

j. Urinary MHPG and Urinary Free Cortisol. Amine metabolites and urinary free cortisol are systematically evaluated using daily 24-hour urine collections across clinical state changes on and off medication.

k. Dexamethasone Suppression Test. Dexamethasone is administered to patients to evaluate the pituitary adrenal axis. Basal values are performed at baseline and at 8 a.m., 4 p.m., and 11 p.m. following dexamethasone administration.

l. Cerebrospinal Fluid and Plasma Studies. Amine metabolites, electrolytes, and peptides are also measured in blood and cerebrospinal fluid.

m. Alpha-Adrenergic Receptors. In collaboration with Dr. M. Kafka, platelet alpha receptor function as well as prostaglandin-stimulated increase in cyclic-AMP are assessed in patients and age-matched normal volunteers.

n. Melatonin. Plasma and urinary melatonin is measured during clonidine and placebo infusions. Clonidine infusions will be administered to panic anxious patients and age-matched volunteers at night during sleep.

o. Glucose and Lactate Metabolism. In collaboration with Dr. B. J. Vittone, clinical and metabolic parameters are evaluated following the oral administration of glucose.

3. Treatment

a. Psychotherapeutic. Treatment and evaluation are conducted in individual and/or group supportive sessions. In addition, ongoing clinical case conferences are utilized.

b. Routine Somatic Treatment. Both routine and experimental compounds are evaluated during double-blind clinical trials. Standard medications used for the treatment of pathological anxiety may be used and include tricyclic antidepressants, monoamine oxidase inhibitors, minor tranquilizers, and beta-blockers.

c. Experimental Compounds. The anticonvulsant carbamazepine and the alpha-2 adrenergic agonist clonidine are in the preliminary stages of investigation as possible treatments of panic and phobic disorders. Alprazolam, imipramine, and propranolol have been administered also to our panic anxious patients in order to assess whether specific biological variables correlate with predicted response to these standard antianxiety agents.

C. Major Findings

1. Medical Illnesses and Pathological Anxiety

Detailed physical, neuropsychiatric, and laboratory evaluations have been performed in approximately twenty-five patients who met Research Diagnostic Criteria for panic disorder. None of these patients had known pre-existing medical illnesses that were thought to be related to their anxiety syndromes. Yet, within this group it has now been established that four patients had evidence of intracerebral pathology (one patient has a deep venous malformation in her right frontal-parietal hemisphere; one patient has complex-partial seizures and a discrete area of decreased uptake deep in her right cerebral hemisphere; one patient has a tremor and dystonic disorder of unknown etiology; and one patient has clear evidence on computerized axial tomography of an old cerebral infarct). These preliminary findings are provocative and suggest the possibility of an increased incidence of and relationship between intracerebral pathology and some conditions of pathological anxiety. In addition, several patients had echocardiographic evidence of mitral valve prolapse, an abnormality previously found to be associated with panic attacks. Furthermore, three patients have been found to have other medical problems, including glomerulonephritis of unknown etiology, multiple endocrine adenomas, and a large uterine tumor. Hypertension is a common finding in patients referred to our program for the evaluation of panic attacks. Several of these patients previously had received incomplete medical workups, perhaps in part because their physical complaints were exclusively attributed by physicians to anxiety. In addition, phobic avoidance may have

contributed to a delay on the part of patients in seeking appropriate consultation and/or treatment.

Together, these preliminary data suggest that patients with severe anxiety require careful medical evaluations for underlying medical diseases which may mimic or exacerbate symptoms of anxiety. Furthermore, some vulnerable patients may have panic attacks triggered by a wide range of different medical illnesses. The common occurrence of hypertension in panic disorder patients may also suggest that patients with labile hypertension may be more vulnerable to the development of panic attacks. Further research is required to determine the prevalence of endocrine, cardiovascular, and neurological diseases in patients with panic and other anxiety-related syndromes. When medical illnesses are present, these patients may require specialized behavioral and/or pharmacologic and/or psychotherapeutic interventions in order for them to obtain appropriate medical care. Without appropriate treatment, some patients may be overwhelmed by phobic anxiety and avoid treatment of even life-threatening illnesses.

2. Life Course of Illness

The life course of illness has been defined in 30 patients referred to NIMH for evaluation and treatment of panic attacks. Eighty-four percent of the patients who met RDC criteria for panic disorder later developed agoraphobia; women were at greater risk than men. Unlike previous reports, the onset of panic attacks in most of our patients was temporally related to circumstances of major stress. Fifty-four percent of the patients with panic disorder had a lifetime history of major depressive symptoms characterized by sadness, crying spells, appetite and sexual disturbance, helplessness, hopelessness, and early morning awakening. Depressive episodes were often brief ($30\% < 2$ weeks) and rarely associated with psychomotor retardation and suicidal ideation. Preliminary data suggest that tricyclic antidepressants block panic attacks in patients with and without concurrent symptoms of major depression, although the presence of depression may confer partial resistance to standard tricyclic antidepressant treatment.

3. Caffeine Consumption and Pathological Anxiety

In collaboration with Dr. J.-P. Boulenger, a survey of both caffeine consumption and its effects on various psychiatric symptoms has been systematically evaluated in patients with anxiety and affective disorders, and normal controls. Thirty patients with panic disorders and their controls matched for age, sex and socio-cultural variables were studied, as well as 23 patients with a history of major depressive disorders and their controls matched in the same conditions. Our data show that in the group of patients with panic disorders but not in their controls a significant positive correlation existed between daily caffeine consumption and the scores of trait-anxiety (as measured by the Spielberger Scale; $r = 0.50$, $p = 0.006$), depression (as measured by the Beck Inventory; $r = 0.56$, $p = 0.02$), and those of 9 out of 13 subscales derived from the SCL-90. No correlation was found between caffeine consumption and any of these measures in the group of patients with major depressive disorders or their respective controls. These results were not related to the consumption of minor tranquilizers, agents which are known to antagonize the effects of caffeine. Thus, our data suggest an increased sensitivity to caffeine in patients with

panic disorder compared to normal controls and to patients with major depressive disorders. This increased sensitivity is consistent with other data in this study demonstrating that patients with panic disorder have an increased responsiveness to one cup of coffee compared to their controls on the following dimensions: anxiety (t-test; $p < 0.01$), activity-alertness (t-test; $p < 0.001$) and sleep (t-test; $p < 0.01$). It is noteworthy that a significantly greater number of patients with panic disorders had given up coffee (67%) when compared to their controls (20%) (chi-square; $p < 0.001$).

Another interesting finding was the trend for patients with major depressive disorders to consume less caffeine when treated with lithium. This finding may be related to the potentiation of lithium side-effects by caffeine and will be more specifically studied in further studies. To document the possible psychopathological and biological effects of caffeine, placebo-controlled caffeine challenges (240, 480, or 720 mg) have been administered to patients with anxiety or major affective disorders and normal volunteers. In the first 5 normal controls studied to date, a dose-effect relationship was found between caffeine and scores of state-anxiety measured by the Spielberger Scale (one way ANOVA; $p = 0.04$).

4. Glucose and Lactate Metabolism

Four panic anxious patients have received a modified five-hour glucose tolerance test. Three patients (75%) developed anxiety and reactive hypoglycemia (glucose less than 55 mg/dl) 3 1/2 - 4 1/2 hours following the administration of glucose. These data may be of importance in understanding the relationship between glucose and lactate metabolism and state anxiety.

5. Clonidine: Probe of Noradrenergic Receptor Function

In an attempt to understand the dynamics of noradrenergic function in depression, we (Drs. Uhde, Siever, Jimerson, Post, and Murphy) evaluated neuroendocrine, biochemical, and cardiovascular responses to the acute intravenous administration of the alpha-2 adrenergic agonist, clonidine 2 µg/kg, in depressed patients and normal controls. Most indices of basal noradrenergic function including plasma norepinephrine (NE) and 3-methoxy-4-hydroxyphenylglycol (MHPG) did not differ between depressed patients and controls, although significantly more variance was observed in the depressed group.

Growth hormone ($p < .05$) and plasma MHPG ($p < .05$) response to clonidine were reduced in the depressed patients compared to the controls, all suggesting reduced responsiveness of alpha-2 adrenergic receptors in depression. Baseline levels of cortisol were elevated in the depressed patients compared to the controls (9.5 ± 5.5 mg/dl, $n = 16$; $p < .05$). Clonidine decreased cortisol to normal levels in the depressed patients but had little effect in the controls. Thus, the depressed patients manifested a significantly increased cortisol response to clonidine. These data raise the possibility that the hypercortisolism of depression may be related to noradrenergic dysfunction.

These results suggest that diminished alpha-2 adrenergic responsiveness as documented by decreased endocrine, biochemical, and physiological responses

to clonidine may be related to the depressive and anxiety symptoms as well as the neuroendocrine disturbances characteristic of many depressed patients.

6. Clonidine as a Treatment for Anxiety

Alterations in noradrenergic function have been postulated in theories of anxiety, fear, and hyperarousal states. Redmond recently proposed a model for the study of anxiety based upon the noradrenergic nucleus locus coeruleus (LC). In animals, electrical or pharmacological activation of the LC produces fear-associated behaviors and increased norepinephrine (NE) turnover, whereas lesions or pharmacological inhibition produces decreased fear-associated behaviors, and decreased NE as well as its metabolite MHPG. In man, urinary, plasma, and CSF MHPG have been correlated with state anxiety.

Clonidine, an alpha-2 adrenergic agonist that inhibits LC activity, reverses the panic anxiety associated with opiate withdrawal and decreases plasma MHPG. These findings suggested to us that clonidine might have antianxiety effects in individuals with pathological degrees of anxiety. In order to explore this hypothesis, our collaborative group (Drs. T.W. Uhde, L.J. Siever, D.C. Jimerson, and R.M. Post) have investigated the behavioral and biochemical effects of the acute intravenous administration of clonidine to 14 depressed, four panic-phobic patients, and 24 normal volunteers. Using the previously validated Spielberger State-Trait Anxiety Inventory (range 20-80), state anxiety was rated at baseline and one hour after clonidine 2 µg/kg or saline infusions. Ten of 14 depressed and all four panic-phobic patients had decreased ratings of anxiety following clonidine compared with baseline. In the combined group of depressed and panic-phobic patients, ratings of anxiety significantly decreased after clonidine (49.1 ± 2.6) compared with baseline (60.7 ± 3.2 ; $n = 18$) and did not change after placebo (pre: 58.2 ± 3.3 , post: 58.1 ± 3.4 ; $n = 11$). In normal volunteers, anxiety following clonidine (pre: 33.5 ± 2.3 , post: 32.2 ± 2.1 ; $n = 24$) or saline (pre: 33.8 ± 2.5 , post: 35.0 ± 2.4 ; $n = 21$) did not differ. This differential antianxiety effect of clonidine in depressed patients and not in normal volunteers was highly significant (2-way ANOVA, $p < .0001$). As predicted by a noradrenergic model of anxiety, clonidine's antianxiety effect was most potent in individuals with the highest baseline values of MHPG ($r = .44$, $p < .05$, $n = 16$, one-tailed).

In collaboration with Drs. J.-P. Boulenger, B. J. Vittone, and R. M. Post, preliminary data from a double-blind, clonidine-placebo crossover trial in six patients with panic disorder also suggest that ratings of generalized anxiety and panic attacks decreased after the first week of oral administration of clonidine. These decrements in anxiety were comparable to the antianxiety effects obtained following the acute, intravenous infusion of clonidine. However, four of our six patients with panic disorder developed tolerance within three weeks of chronic clonidine administration. The loss of anxiolytic effects with chronic clonidine treatment parallels the time course and development of tolerance to the inhibiting effects of clonidine on the firing rate of the LC in animals observed by others. In addition to tolerance, clonidine treatment may be associated with a number of untoward side effects such as drowsiness, sedation, and dry mouth. The role of the LC in fear-related behaviors, arousal, and the sleep-waking cycle may help explain the common association between anxiety

reduction and sedation for most of the anxiolytic drugs, e.g., benzodiazepines, barbiturates, and meprobamate.

The results of these preliminary studies suggest that clonidine may have noteworthy antianxiety effects in depressed and phobic and panic anxious patients, as well as patients undergoing opiate withdrawal. In addition, our preliminary data suggest that independent measures of noradrenergic activity may be related to clonidine's antianxiety properties. Although clonidine may be less well tolerated by anxious patients than non-anxious hypertensives or patients undergoing opiate withdrawal, double-blind comparisons of clonidine to standard drug treatments, e.g., imipramine, are indicated.

7. Clonidine as an Analgesic Agent

The potential use of clonidine as a nonopioid, nonaddicting analgesic agent is of interest since clonidine has antinociceptive effects greater than or equal to morphine in animals and both blocks and reverses opiate withdrawal in man. The acute effects of clonidine 2 µg/kg on psychophysical pain has been assessed in 14 normal volunteers. Using threshold and signal detection analysis, preliminary data indicate that clonidine lacks analgesic activity in man as measured by the index of pain insensitivity. However, there was a significant association between fall in blood pressure and increased insensitivity ($r = 0.71$, $n = 12$, $p < .01$). The fact that the subjects with the greatest fall in blood pressure had increased pain insensitivity may suggest that, within individuals, dose-response effects of clonidine may alter nociceptive thresholds in man. Furthermore, in a subgroup of pain-insensitive individuals, clonidine produced changes on evoked response consistent with an analgesic effect. This research, accomplished in collaboration with Dr. M.S. Buchsbaum, suggests that individual differences in baseline pain sensitivity might predict patients' antinociceptive response to clonidine.

8. Clonidine and Plasma Melatonin

The effect of clonidine on plasma melatonin during sleep has been studied in collaboration with Drs. A. Lewy and L.J. Siever. Clonidine 2 µg/kg i.v. produced at least a 50% reduction in plasma melatonin in all normal controls. This preliminary finding is noteworthy and provides a unique methodology by which noradrenergic responsivity in anxious patients may be assessed.

9. Yohimbine Challenge

Yohimbine is a relatively selective alpha-2 adrenergic antagonist which acts at the presynaptic level to enhance, rather than reduce, the neuronal release of norepinephrine in the central nervous system. Yohimbine can also activate central noradrenergic systems at low doses which do not have peripheral or post-synaptic effects in animals. In animals, yohimbine may increase behaviors related to fear and has some of its effects antagonized by diazepam. In man, previous investigations found that yohimbine induced anxiety and autonomic symptoms such as tachycardia and sweating in various groups of psychiatric patients when given at high doses. However, yohimbine given orally at lower doses has not been found to be associated with significant untoward

side effects in psychiatric patients. In collaboration with Drs. J.-P. Boulenger, B. J. Vittone, and R. M. Post, a low-dose yohimbine (5-20 mg p.o.) oral challenge paradigm has been initiated to test the hypothesis of an increased noradrenergic sensitivity in patients with panic attacks compared to normal controls and affectively ill patients without panic attacks. Preliminary data suggest that yohimbine induces panic attacks in patients with panic disorder. Studies with yohimbine may provide an advantage over other agents such as lactate which has been used as a provocative test in anxious patients, but whose mechanism of action is less clearly specified than that of yohimbine.

10. Anxiety and Psychophysical Pain

The subjective experiences of anxiety and pain are prevalent symptoms in psychiatric patients. In addition, anxiety is usually thought by clinicians to enhance pain appreciation. Several authors even have suggested that anxiety intensifies psychophysical pain by directing attention toward pain sensations. Furthermore, alterations in noradrenergic function have been implicated in the modulation of both anxiety and pain. In an attempt to clarify these variables, we have investigated the relationship among anxiety, psychophysical pain, and plasma-free MHPG in 12 normal volunteers.

In collaboration with Drs. M.S. Buchsbaum and D.C. Jimerson, we have demonstrated significant correlations between state anxiety and scores on the insensitivity index ($r = .67$, $n = 12$, $p < .05$) and state anxiety and plasma-free MHPG ($r = .59$, $n = 12$, $p < .05$). Thus, the subjects with the highest ratings of state anxiety had the greatest plasma-free MHPG and were least able to discriminate between distinct and unpleasant sensations. By median split, the six most pain insensitive individuals had significantly higher levels of plasma-free MHPG ($3.2 \text{ ng/ml} \pm 0.2 \text{ S.E.}$) and anxiety (39.1 ± 1.8) compared to the six least pain insensitive individuals (MHPG 2.5 ± 0.1 , $p < .20$; anxiety 27.8 ± 1.6 , $p < .001$).

These findings represent one of the first reports to suggest that high anxiety may reduce, rather than enhance, the ability to discriminate the amount of pain a patient experiences. In some circumstances, therefore, treating pain patients with anxiolytics might paradoxically intensify the amount of pain experienced as measured by the insensitivity index.

11. Anxiety and Sleep Architecture

Insomnia is commonly believed to result from anxiety or other states of increased autonomic arousal. In accordance with this hypothesis many clinicians have employed relaxation techniques, biofeedback, systematic desensitization, and antianxiety pharmacotherapy in the treatment of insomnia. Although anxiety is a frequent concomitant of insomnia, no laboratory has investigated either the prevalence of insomnia or the sleep architecture of patients who meet Research Diagnostic Criteria (RDC) for the panic and phobic disorders. Furthermore, a comparison of sleep between panic anxious and depressed patients, as well as normal controls, is indicated since both panic anxious and depressed patients respond to tricyclic and MAO inhibitor drugs. In collaboration with Drs. J.C. Gillin and W. Mendelson, we are investigating both the frequency of complaints of insomnia and the sleep architecture of patients who meet RDC for panic or

phobic disorders. Preliminary evidence suggests that many of the panic anxious patients do not report subjective sleep disturbance. In fact, several of the patients stated that the subjective experience of restful sleep was associated with less daytime anxiety and reduced number of panic attacks after awakening. While these particular patients "looked forward" to sleep onset, they also recorded numerous sleep-incompatible and avoidance behaviors, and often delayed retiring to their rooms until after midnight. In relationship to sleep architecture, there was no significant difference between anxious patients and the published norms of age-matched normal volunteers in any of the following criteria: early morning awakening, sleep efficiency, intermittent awake time, delta sleep, and REM density. Preliminary findings suggest, however, that the panic anxious patients as a group had significantly reduced REM latency and less total sleep time, even though they did not experience subjective sleep disturbance. The finding of decreased REM latency in this population is noteworthy in relationship to other tricyclic-responsive disorders such as Major Affective Illness and Obsessive-Compulsive Disorder which are also associated with decreased REM latency.

12. Alpha-Adrenergic Receptors

In collaboration with Dr. M. Kafka, alpha-adrenergic function was measured in the platelets of patients with panic disorder and in age- and sex-matched normal controls. ^3H -dihydroergocryptine ($^3\text{HDHE}$) binding was increased, whereas prostaglandin E_1 (PGE_1)-stimulated cyclic AMP (cAMP) production was decreased in patients compared to controls. The percent inhibition by norepinephrine of PGE_1 -stimulated cyclic AMP was lower in patients than controls. Similar alterations in alpha-adrenergic function have been found in patients with major depressive illness. These data suggest that similar alterations in noradrenergic function may occur in the panic and affective disorders.

D. Proposed Course of Project: Research conducted by the Unit on Anxiety and Affective Disorders has demonstrated a blunting of the clonidine-induced growth hormone (GH) response in affectively ill patients compared to age- and sex-matched controls. These findings, which have been replicated by four independent research groups, may suggest decreased postsynaptic noradrenergic function in endogenous depression.

Increasing evidence also suggests a heterogeneity in anxiety and depressive syndromes. Thus, the neuroendocrine (GH) response to clonidine in patients with anxiety disorders (with and without depression) may provide a useful methodology for elucidating the biological relationships of various anxiety disorders to each other as well as to depression. We plan to continue the yohimbine challenge study to further assess noradrenergic function in patients with pathological anxiety and depression. Diazepam, a standard antianxiety agent, will also be given to patients and controls to investigate the relationships among anxiety, psychophysical pain, and various peripheral correlates. We also intend to continue our studies with caffeine. Further delineation of the clinical response to caffeine is indicated because caffeine consumption is correlated with symptoms of generalized anxiety in patients with panic attacks, but not in normal volunteers. Caffeine derivatives also activate noradrenergic activity in animals when iontophoretically applied to the LC. Furthermore, caffeine has

been shown by others to antagonize the biochemical and pharmacological effects of benzodiazepines in humans. This study, therefore, will represent an initial attempt to investigate the effect of caffeine on mood, anxiety, and behavior in clinically distinct patient groups. Furthermore, caffeine has been reported to induce arrhythmias in patients with mitral valve prolapse (MVP), a syndrome also associated with anxiety. The relationship among caffeine sensitivity, MVP, anxiety and arrhythmias will be investigated.

Studies of the clinical efficacy of alprazolam, clonidine, carbamazepine, imipramine, and propranolol in panic and phobic patients will be continued. Our preliminary research with clonidine in depressed and anxious patients and normal volunteers is encouraging and suggests that clonidine might be especially useful in patients who experience context-dependent, e.g. anticipatory anxiety. In addition, alprazolam, carbamazepine, and imipramine will be studied with special emphasis on its potential usefulness as a prophylactic agent in blocking spontaneous panic attacks. The clinical and biological predictions of drug response will also be investigated in anxious and affectively-ill patients. These clinical trials, in conjunction with concomitant measurements of the neurotransmitter effects, should enhance our understanding of alterations in neurotransmitter pathways associated with pathological anxiety and its amelioration with appropriate psychopharmacotherapies.

E. Significance to Biomedical Research and the Program of the Institute

Several epidemiological surveys have suggested that pathological degrees of anxiety may adversely influence a large segment of our population. Agoraphobia, an anxiety syndrome associated with "spontaneous" panic attacks, results each year in the impairment of individuals previously well-functioning and productive. Furthermore, the role of anxiety and stress in coronary heart disease has been suggested by a number of studies. Moreover, emerging epidemiological and familial data suggest that a subgroup of patients with major depressive illness plus panic attacks may represent an important and distinct subtype of major affective illness. We intend to investigate biological correlates in the plasma and cerebrospinal fluid of this subtype, who may be at greater risk for alcoholism and suicide, compared to patients with major depressive illness without panic attacks. An improved understanding of the clinical and biological aspects of both normal and pathological anxiety is thus critically needed. It is hoped that the developing battery of clinical and biological tests in patients with anxiety and related mood disorders will ultimately provide a clinical and biological profile of these illnesses and lead to more refined subcategorizations, as well as to more selective and efficacious treatment approaches.

Selected References

Uhde, T.W., Redmond, D.E., Jr., and Kleber, H.D.: Psychosis in the opioid addicted patient: assessment and treatment. J. Clin. Psychiatry 43: 240-247, 1982.

Uhde, T.W., Boulenger, J.-P., Siever, L.J., DuPont, R.L., and Post, R.M.: Animal models of anxiety: implications for research in humans. Psychopharmacol. Bull. 18: 47-52, 1982.

Uhde, T.W., Siever, L.J., Post, R.M., Jimerson, D.C., Boulenger, J.-P., and Buchsbaum, M.S.: The relationship of plasma free MHPG to anxiety and psychophysical pain in normal volunteers. Psychopharmacol. Bull. 129-132, 1982.

Uhde, T.W. and Post, R.M.: Effects of carbamazepine on serum electrolytes: clinical and theoretical implications. J. Clin. Psychopharmacol. 3: 103-106, 1983.

Boulenger, J.-P. and Uhde, T.W.: Peripheral biological correlates of anxiety. L'Encephale 8: 119-130, 1982.

Boulenger, J.-P. and Uhde, T.W.: Aspects of biochimiques de l'anxiety. Le Semaine des Hopitaux 58: 2573-2579, 1982.

Boulenger, J.-P. and Uhde, T.W.: Caffeine consumption and anxiety: preliminary results of a survey comparing patients with anxiety disorders and normal controls. Psychopharmacol. Bull. 18: 129-132, 1982.

Uhde, T.W., Siever, L.J., and Post, R.M.: Clonidine: acute challenge and clinical trial paradigms for the investigation and treatment of anxiety disorders, affective illness, and pain syndromes. In Post, R.M. and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins, in press.

Siever, L.J. and Uhde, T.W.: New studies and perspectives on the noradrenergic receptor system in depression: effects of the alpha-2 adrenergic agonist clonidine. J. Biol. Psychiatry, in press.

Uhde, T.W., Roy-Byrne, P., Boulenger, J.-P., Vittone, B., Geraci, M., and Post, R.M.: The relationship between panic disorder and major depressive illness. Prog. Neuropsychopharmacol. Biol. Psychiatry, in press.

Boulenger, J.-P. and Uhde, T.W.: Le traitement des attaques de panique. L'Encephale, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 MH 00072-03 BP

PERIOD COVERED

October 1, 1982 to September 1, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Psychophysiological Investigation of Multiple Personality Disorder

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Frank W. Putnam, M.D., Staff Psychiatrist, Adult Psychiatry Branch

COOPERATING UNITS (if any)

Nursing Dept., 3-West, NIH

Clinical Neuropharmacology Branch, NIMH

Laboratory of Psychology and Psychopathology, NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychobiology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2

PROFESSIONAL:

2

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project investigates the syndrome of multiple personality disorder (MPD). The investigation continues to focus on three major areas. The first is the physiologic and neurophysiologic differences reported to exist among the alternate personality states. The existence of certain types of differences has been documented and further research is underway to elucidate the mechanisms of these state-related changes. The second is on the symptoms, presentation, and phenomenology of the clinical syndrome. An extensive case finding study is underway, sampling cases in treatment in the United States and abroad. The third focus is on a prospective study of cases currently in treatment to clarify the long-term outcome of this unusual disorder.

Psychophysiological Investigation of Multiple Personality Disorder

I. Project Description

A. Objectives

This study is a multi-disciplinary effort to investigate and rigorously document the clinical and physiological phenomena of the multiple personality disorder syndrome and related dissociative states. There are three major lines of investigation into this unusual disorder.

1. The first approach utilizes repeated neurophysiological measures across alternate personalities which are compared to control subjects simulating this condition.

2. The second line of inquiry is the establishment of a large scale data base of cases collected in a standardized manner. A 24 page questionnaire is used to collect data on: presenting symptoms, past psychiatric, family, social, childhood and educational history; method of diagnosis, phenomenology of the alternate personalities treatment and outcome.

3. The third avenue of investigation are prospective studies on a cohort of patients undergoing treatment around the United States. Several cases of multiple personality disorder in children and adolescents are included in this cohort of patients.

B. Methods Employed

1. Subjects

a. Patients who meet DSM III criteria for multiple personality disorder are admitted as outpatients to the NIH clinical center under protocol 80-M-142. These patients serve as subjects in the physiological and psychological investigations.

b. Patients in Maryland, Virginia, and the D.C. area, as well as selected patients involved in the physiological investigations are screened and interviewed for participation in the long-term studies.

c. Clinical center normal volunteers and professional actors affiliated with the Psychodrama Institute located at St. Elizabeths Hospital in Washington, D.C. serve as control subjects for the physiological and psychological studies.

2. Neurophysiological Investigations

a. Drug-free subjects undergo a repeated series of EEG and visual evoked potential studies in collaboration with Dr. John Morihisa of the Adult Psychiatry Branch.

b. Galvanic skin response (GSR) and other autonomic measures are being studied across alternate personality states and simulating controls in

collaboration with Dr. Theodore Zahn of the Laboratory of Psychology.

c. Cerebral blood flow using the xenon inhalation technique is being studied across alternate personality states tested under two conditions: a resting study and a activated study using an automated version of the Wisconsin Card Sort. These studies are being conducted in collaboration with Dr. Daniel Weinberger and Dr. Ronald Zec of the Adult Psychiatry Branch. Professional actors simulating multiple personality patients are serving as controls.

3. Questionnaire Studies

A 24 page (186 item) questionnaire developed and piloted during 1981-1982 has been distributed to clinicians around the United States who are engaged in treating patients with multiple personality disorder. Presently, over 150 cases have been collected and analyzed with this form. A very large scale sampling questionnaire is currently under development which will be used to determine the incidence and prevalence of multiple personality disorder in the U.S.

4. Prospective Studies

a. The first prospective study involves the follow up of a cohort of adult multiple personality patients undergoing a variety of treatment modalities. These patients were all screened with a standardized, videotaped interview and are being followed at one year intervals.

b. The second group of patients involved in prospective studies are children identified by two local sexual abuse agencies - The Prince Georges County Sexual Assault Center and the Montgomery County Protective Services. These children fit a profile developed through the questionnaire study and a literature review and are followed by social workers in the respective agencies. The focus on sexual abuse derives from the questionnaire study data showing that 83% of adult multiple personality patients suffered sexual abuse as children.

C. Major Findings

1. Neurophysiology

a. Visual evoked potential studies demonstrate personality specific changes which are not matched by simulating control subjects.

b. Power spectral analysis of the EEG data show statistically significant differences in the high frequency Beta waves across alternate personalities that are not produced by simulating control subjects.

c. Proactive inhibition memory testing reveals a statistically significant higher discrimination of set in multiple subjects compared to simulating controls. In short, these findings indicate that while learning a second list of words increases intrusion errors in the first list in normals, less intrusions occur in across personalities in multiples, supporting their claim of amnesia across personalities.

2. Questionnaire Study

a. An analysis of 100 current cases of multiple personality studied in collaboration with R. Post, M.D., J. Guroff, E. Silberman, and L. Barban, reveals a high degree of similarity in symptoms and alternate personality structure across patients. This data was presented at the APA in the New Research Section. The most striking finding is the high incidences of reported child abuse suffered by these patients. The constellation of symptoms commonly present at initial evaluation included: depression (88%), suicidality (68%), headaches (66%), amnesic episodes (57%), fugue spells (55%), panic attacks (54%), depersonalization (53%), and unexplained physical pain (46%). Frequent previous psychiatric diagnoses include depression (70%), personality disorders (49%), and schizophrenia (47%). The patients were largely female (92%) with a mean age at diagnosis of 31.3 years and an average of 13.3 (range 1-60) alternate personalities. In 80% of the cases the personality presenting for treatment was unaware of the existence of alternate personalities. The diagnosis was most commonly made after an alternate personality presented to the therapist (51%). Common alternate personality types include: child personalities (77%), a memory trace (75%), and opposite-sex alternates (55%). Differences observed among the alternates included different physical symptoms (74%), differential response to medication (46%) and different handedness (37%). In 97% of the cases there was history of a traumatic childhood, with sexual abuse (83%), physical abuse (75%), extreme neglect or abandonment (61%) and witnessing extreme violence (41%) among the more commonly reported traumas. Significant correlations included the total number of types of childhood trauma with the number of alternate personalities ($p < .007$) and the total number of psychopathologic symptoms with the number of alternate personalities ($p < .007$). Hypnotherapy and psychotherapy were the modalities rated most effective in both short and long term treatment. A cluster analysis of symptom categories suggests a number of subtypes of MPD patients with different treatment outcome.

3. Prospective Studies

a. No findings are currently available at this time. Approximately 30 patients and therapists are participating in the adult study and 8 children and adolescents are involved in the child study.

D. Implications of the Research

The physiological studies confirm the anecdotal reports of clinicians over the last 150 years who noted physiological differences across alternate personality states. These replicable physiological changes may provide clues as to the interaction of personality and physiology which will be useful in understanding psychosomatic processes.

The questionnaire data is providing the first large scale data on the nature of this disorder and demonstrates that there is a high degree of similarity across cases for most phenomena of this disorder. The linkage to child abuse has often been suspected, but the questionnaire data confirms that multiple personality disorder is highly correlated with child abuse.

The clinical profile developing from the above studies will prove useful in aiding clinicians to recognize and treat dissociative phenomena.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00180 - 01 BP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiology and Treatment of Menstrually-Related Mood Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David R. Rubinow, M.D., Chief, Unit on Peptide Studies, BPB, NIMH		
COOPERATING UNITS (if any) <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> Psychobiology Branch, NIMH Hypertension-Endocrine Branch, NHLBI Clinical Pathology Department, CC </div> <div style="width: 50%;"> Endocrinology & Reproduction Res. Br., NICHD Lab. of Psychology & Psychopathology, NIMH </div> </div>		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neuroendocrinology		
INSTITUTE AND LOCATION NIMH - N.I.H. Bethesda, Maryland 20205		
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The occurrence of dramatic changes in mood, behavior, cognition and somatic functioning in some women in relation to the menstrual cycle has recently been the focus of a great deal of public scrutiny. Yet despite fifty years of study, relatively little is known about the relationship between menstruation and disorders of mood. This project addresses itself to the major methodological difficulties characterizing earlier studies and is designed to study the psychobiology and treatment response of women with well defined <u>menstrually-related mood disorders</u>. The longitudinal screening methods employed in the first phase of our study appear capable of distinguishing women with <u>menstrually-related mood syndromes</u> from those who only believe that they have such a syndrome. We are currently measuring potential biological correlates of <u>menstrually-related mood changes</u> by assaying serial blood samples for relevant hormones and by performing neuroendocrine and electrophysiological tests during the symptom-free and symptomatic phases of the menstrual cycle. We are additionally performing double-blind controlled studies of several putative therapeutic agents including progesterone and pyridoxine. </p> <p> The goals of this project are to detect and accurately describe <u>menstrually-related mood disorders</u>, explore their pathophysiology and response to pharmacological and environmental manipulation, and to document the relationship between reproductive endocrine change and disorders of mood as a way of further investigating the neurobiology of psychiatric illness. </p>		

I. Project Description

A. Objectives

This project has as its main intent the selection of subjects with carefully documented menstrually-related mood changes who can then undergo psychological and biological evaluation as well as participate in double-blind, placebo-controlled trials of several widely prescribed treatment modalities.

B. Methods Employed

1. Subjects

a. Subjects are self- and physician-referred women between the ages of 18 and 55 who complete visual analogue scale mood ratings twice daily for two months and on the basis of these ratings meet the following operational definition: a greater than 30% increase in self-rated anxiety or depression during the week prior to menses compared to the week following the cessation of menses in at least two consecutive cycles. All study participants are outpatients admitted to the outpatient division of the Section on Neuroendocrinology, Biological Psychiatry Branch.

b. Normal controls for this study include women with no complaints nor evidence of menstrually-related mood disorder and who are without primary psychiatric illness, and women who have complaints of, but no visual analogue scale evidence of, menstrually-related mood changes.

2. Procedures

Phase 1. The initial phase of this study is a screening phase involving subjects who are self- or physician-referred with complaints of severe changes in mood in apparent relation to the menstrual cycle. All subjects complete an extensive screening form which assesses the frequency and severity of symptoms in relation to menstruation as well as past and present psychiatric history, social history, family history, medical and GYN history, and medication history. Subjects are also provided with three months' worth of visual analogue scales for anxiety and depression which they are asked to complete on a twice-daily basis. Finally, subjects are individually interviewed in order to more adequately assess menstrually-related phenomenology, medical history, and psychiatric history, with all patients administered the schedule for affective disorders and schizophrenia interview in order to produce a lifetime psychiatric diagnosis.

Phase 2. This is an intensive psychobiological evaluation phase for patients meeting entry criteria for the study.

a. Patients are given a thorough physical and laboratory examination in order to rule out the presence of unknown medical illness.

b. Plasma steroid and peptide studies. Fasting 8:00 a.m. blood samples are obtained at nine points during a menstrual cycle in order to evaluate the levels, relative concentrations, and pattern of secretion of several peptide and steroid hormones which have been implicated in menstrually-related mood dis-

orders such as estrogen, progesterone, aldosterone, and beta-endorphin. These studies are being performed in collaboration with Drs. George Merriam (ERR, NICHD) and Philip Gold (BPB, NIMH).

c. Plasma catecholamines. Because of the putative role of central catecholaminergic function in affective disorder and because of evidence that plasma norepinephrine concentrations vary in synchrony with the menstrual cycle, we are measuring plasma norepinephrine, epinephrine, and DOPAC in collaboration with Dr. David Goldstein (HE, NHLBI).

d. Plasma magnesium. Because of reports of altered monocyte magnesium levels in women with "premenstrual tension," we are measuring plasma monocyte magnesium concentrations at two points during both the follicular and luteal phases of the menstrual cycle in collaboration with Dr. Ronald Elin (CP, CC).

e. Additional neuroendocrine measures. In order to assess whether specific neuroendocrine abnormalities accompany the symptomatic phase in women with menstrually-related mood disorders, we are performing two well described (dexamethasone suppression test and TRH stimulation test) and one recently described (CRH stimulation test) neuroendocrine tests employed in affective disorder studies. The dexamethasone suppression test and TRH stimulation test are both performed in routine fashion during both the symptom-free and symptomatic phases of the menstrual cycle. The corticotropin releasing hormone (CRH) stimulation test is performed in collaboration with Dr. Philip Gold and involves administering five micrograms per kilogram of CRH in bolus form followed by two hours of periodic blood sampling.

f. Electrophysiological measures. Because of the prominent central nervous system effects of many of the reproductive hormones which vary in their concentration over the course of the menstrual cycle and because of frequent reports of altered responsivity to stimuli during the premenstruum, we are measuring galvanic skin response and other autonomic measures during the symptomatic and and symptom free phases of the menstrual cycle. These studies are being performed in collaboration with Drs. Theodore Zahn (LPP, NIMH) and Renate DeJong (CP, NIMH). We are additionally attempting to investigate the oft-reported premenstrual cognitive dysfunction by examining the P-300 component of the average evoked response in collaboration with Dr. Connie Duncan-Johnson (LPP, NIMH).

g. Sleep measures. In collaboration with Dr. Barbara Parry (CP, NIMH) we are obtaining electroencephalographic sleep recordings during three consecutive nights at four points during the menstrual cycle. Additionally, activity measure are obtained utilizing the activity monitor developed by Dr. Theodore Colburn (RSB, NIMH).

h. Psychometrics. In addition to conventional self-ratings of anxiety, depression, and mood during blood sampling days, each patient completes on a twice-daily basis computer scanable visual analogue scales for depression, anxiety, fatigue and global assessments and keeps a sleep log on a daily basis. A twenty-one item assessment form developed by Dr. Jean Endicott specifically for patients with menstrually-related disorders is being used by our patient group. In collaboration with Dr. Gerald L. Brown (BPB, NIMH), MMPI's are completed by all patients in the second phase of the study. Records of frequency and perception of

stressful events are being completed under the supervision of Dr. Renate DeJong, and a method for assessing life events and external stressors has been developed by Dr. Peter Roy-Byrne (BPB, NIMH). Cognitive batteries to be administered during symptomatic and symptom-free phases have been assembled by Christine Hoban (BPB, NIMH).

Phase 3. This is a multi-modality treatment phase for patients who have completed Phase 2.

a. Pharmacologic. Double-blind, placebo-controlled crossover evaluations of progesterone, medroxyprogesterone acetate, pyridoxine, and carbamazepine are either planned or currently being conducted. The first three agents mentioned are cited as effective in the literature but have not been systematically demonstrated to be more effective than placebo in studies to date, largely as a function of methodological flaws which render the results of these studies ungeneralizable. Carbamazepine has been successfully used in the treatment of premenstrual psychomotor seizure-related behavioral syndromes as well as major affective disorder.

b. Sleep deprivation. In collaboration with Dr. Barbara Parry we plan to evaluate sleep deprivation as a therapeutic modality in patients with premenstrual mood disorders. In addition to its therapeutic potential, this procedure may also yield information that may help to clarify the relationship between menstrually-related mood disorders and major affective disorders.

C. Findings

This study has been ongoing since October 1982, and thus most of the major findings to date relate to the first phase of the study. Our most significant accomplishment to date has been the demonstration of the effectiveness of the visual analogue scale prospective rating methods in the discrimination of women with and without menstrually-related disorders. Approximately 40% of the first 60 women to complete their visual analogue scales had clear evidence of a menstrually-related mood syndrome. These women for the most part were historically and clinically indistinguishable from those lacking evidence of a menstrually-related mood syndrome, although a comparison of the responses of these two groups on the initial screening questionnaire has not yet been performed.

D. Proposed Course of Project

To date over 500 women have requested to be participants in our project and are at various stages of evaluation. With a group of well defined patients, we hope to explore the natural course of menstrually-related mood disorders as well as their phenomenology and biological correlates in relation to treatment response. In addition we wish to expand our investigation of the effects of menstrual phase on mood to include patients hospitalized at the Clinical Center with major affective disorder, panic anxiety disorder, anorexia-bulimia as well as patients with hereditary angioedema. Our early experience with a number of women with these disorders suggests that symptoms may be exacerbated or may cluster during the premenstruum period; these clinical impressions require prospective confirmation.

E. Significance to Biomedical Research and the NIMH Intramural Research Program

Despite the current lack of clear understanding of the nature of the relationship between mood disorders and the menstrual cycle, numerous studies of this phenomenon suggest its importance to the psychiatrist on many levels: practically (as a problem about which the psychiatrist may be called to consult or as a factor which may influence the course of the treatment of patients); heuristically (as a model for learning about state changes, a process of clear relevance to studies of other mood state disorders such as manic-depressive illness or panic anxiety disorder); and conceptually (as a potential means for providing biological-phenomenological isomorphs and further understanding the role of entrainment in episodic or cyclic psychiatric disorders). Menstrually-related mood disorders in their own right are important to better understand, if only for the fact that there are large numbers of women who feel that they suffer from such syndromes and seek treatments which are unproved and potentially dangerous. In addition it would appear that menstrual cycle phase is a variable which has been all too frequently ignored in studies of traditional psychiatric and medical illnesses. It is our belief, therefore, that this project will provide information that will be of immediate clinical relevance and that will further our understanding of the complex relationship between endocrine system activity and mood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00452-08 BP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Neuroendocrine Studies of Major Psychiatric Disorders		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Philip W. Gold, M.D.		
COOPERATING UNITS <i>(if any)</i> Tufts Univ., Boston, Mass.; Developmental Endocrinol., NICHD; Univ. of Chicago, Chicago, IL; Experimental Ther. Branch, NICHD; Surgical Neuroendocrinol. Branch, NINCDS, NIH.		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, Bethesda, MD 20205		
TOTAL MANYEARS: 5.4	PROFESSIONAL: 3.0	OTHER: 2.4
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>Current studies concentrate on comprehensive tests of hypothalamic-pituitary function and on the measurement and administration of endogenous neuropeptides or their analogs. Major findings this year are: the plasma half-life of corticotropin releasing factor (CRF) in humans is greater than for any other known endogenous hypothalamic hormone. Administration of $\mu\text{g/kg}$ of CRF at 20:00 h constitutes a clinically applicable test of the human hypothalamic-pituitary-adrenal (HPA) axis. For instance, in contrast to normal volunteers, drug-free bipolar subjects, regardless of clinical state, show a significant growth hormone response to CRF. In addition, drug-free bipolar depressed patients show blunted ACTH responses to CRF, indicating normal feedback regulation of cortisol on ACTH secretion at the pituitary, and suggesting increased hypothalamic CRF secretion as the basis for hypercortisolism in depression. To support this hypothesis, continuous CRF infusion to normals produced hypercortisolism of the magnitude typically seen in depression. Patients with Cushing's disease, in contrast to depressed patients, show a robust response to ACTH despite high baseline cortisol values, reflecting a marked impairment of feedback regulation at the pituitary. Thus, the responses to CRF suggest that the pathophysiologic locus of HPA regulation is different in depression and Cushing's disease and the CRF stimulation test may aid in the differential diagnosis of the two entities.</p>		

Collaborators:

Dr. L. Loriaux, Chief, Developmental Endocrinology Branch, NICHD

Dr. G. Robertson, Professor of Medicine, University of Chicago

Dr. E. Oldfield, Senior Investigator, Surgical Neurology Branch, NINCDS

Dr. G. Chrousos, Senior Investigator, Developmental Endocrinology Branch, NICHD

Dr. C. Kellner, Medical Staff Fellow, Biological Psychiatry Branch, NIMH

Dr. R.M. Post, Chief, Biological Psychiatry Branch, NIMH

Dr. D. Rubinow, Chief, Unit on Peptide Studies, Biological Psychiatry Branch, NIMH

Dr. S. Reichlin, Chief, Division of Endocrinology, Tufts University

Dr. G. Merriam, Senior Investigator, Endocrinology Branch, NICHD

Project Description:

Objectives: Several aspects of the symptom complex of the functional psychoses, particularly affective illness and anorexia nervosa, suggest hypothalamic dysfunction. For instance, patients with depression or mania often show disturbances in sleep, altered energy levels, changes in appetite and libido, diurnal variation in symptoms, alterations in the consolidation of memory traces, and changes in reproductive function such as amenorrhea. Anorexics show not only profound alterations in appetitive behavior, but also marked functional changes in the hypothalamic-pituitary axis, including abnormalities in gonadotropin, thyrotropin, growth hormone and vasopressin secretion. Interest in the hypothalamic-pituitary axis has also been stimulated by recent findings that the monoaminergic neurotransmitters modulate the synthesis and release of a number of hypothalamic peptides and pituitary hormones. Thus, examination of pituitary hormones in plasma can shed light on the functional activity of biogenic amine systems. Moreover, the hypothalamic hormones themselves have been shown to be widely distributed in brain, exert specific receptor mediated biological actions, and influence the functional activity of brain neurotransmitter systems. Several hypothalamic hormones have also been shown to have profound effects on complex behaviors and cognition.

In our clinical studies, several neuroendocrine strategies have been routinely utilized: (1) direct measurement in the cerebrospinal fluid (CSF) and in the plasma of behaviorally active peptides during the basal state and/or following stimulation according to verified stimulation paradigms; (2) administration of hypothalamic releasing factors to test responses of the hypothalamic-pituitary axis and to elucidate patterns of monoaminergic disturbance and neuroendocrine dysfunction; (3) effects of psychoactive drugs on hypothalamic-pituitary function and on the levels of behaviorally active peptides; (4) assessment of the temporal organization of neuroendocrine function; (5) assessment of the relationship between neuroendocrine function and sleep.

For purposes of comparison and possible differential diagnosis, normal subjects, patients with affective illness, schizophrenia, anorexia nervosa, and Cushing's disease are studied. Our group is also actively involved in studying the neurobiology of several neuroendocrine diseases, particularly Cushing's syndrome, as well as Kallman's disease, and in developing clinical means for the differential diagnosis of Cushing's disease from depression and for distinguishing hypothalamic from pituitary Cushing's disease. In addition to these clinical studies, we have studied neurohormonal function in stalk-sectioned and intact subhuman primates to examine dose-response relationships, pharmacokinetics, and the physiology of peptide secretion into the plasma and CSF spaces.

The Unit on Neuroendocrinology has established an extensive collaborative clinical and laboratory relationship with the Developmental Endocrinology Branch of the NICHD headed by Dr. Lynn Loriaux. Clinically, the project addresses possible pathophysiological analogies between depression and Cushing's syndrome, with a focus on developing a clinical test useful in the differential diagnosis of the two entities. A major accomplishment of this collaboration and of our work this year has been on the development of a clinically applicable new test of ACTH secretion, namely, a corticotropin releasing factor (CRF) stimulation

test. In the laboratory, we have developed radioimmunoassays for a variety of peptide hormones, including ACTH, beta-endorphin, alpha-MSH, vasopressin, oxytocin, and CRF. This project will be discussed in detail below.

Methods Employed:

(1) Studies of behaviorally active CNS peptides:

a. Corticotropin Releasing Factor (CRF): A major effort of our work this year has been on basic and clinical studies of the newly sequenced peptide CRF, a 41 amino acid peptide with greater ACTH releasing potency than any other previously identified endogenous or synthetic peptide in pituitary cultures in vitro and in vivo. The sequencing of CRF provides the most direct opportunity so far to study central control of the hypothalamic-pituitary-adrenal (HPA) axis and coincides with increasing interest in the regulation of the HPA axis in patients with primary affective disorder. Moreover, CRF, like many other hypothalamic peptides, is widely distributed in brain and may play an important role in orchestrating a variety of physiological and behavioral events classically associated with response to stress.

b. Studies of CRF in Sub-human Primates: CRF given as a bolus to stalk-sectioned and intact cynomolgus macaques causes a dose-dependent increase in plasma cortisol secretion. The middle of the dose-response curve is between 0.5 and 2 ug/kg with the peak cortisol response occurring after 15-30 minutes at low doses (0.5 ug/kg) and after 45-90 minutes at higher doses (2-40 ug/kg). The plasma cortisol responses to CRF remain elevated for the entire three hour period of plasma sampling. The dose-response curve for CRF induced cortisol secretion is similar to that of the TSH response to TRH and of the LH response to LH-RH.

Radioiodinated CRF given to cynomolgus macaques as an i.v. bolus injection disappears from plasma in a biexponential fashion with a short half-life of 17 ± 3 minutes and a long half-life of 19 ± 5 minutes. These half-lives are considerably longer than those of any other hypothalamic releasing factor such as LH-RH or TRH. This relatively prolonged half-life could account for the prolonged elevation in plasma cortisol (at least 3 hours) seen in stalk-sectioned and intact primates after bolus injection. As expected, the metabolic clearance rate of CRF, measured by the steady state infusion method, was considerably longer than for any other hypothalamic hormone. For instance, the metabolic clearance rate of CRF is only 10-20% that of ACTH. No CRF was found in CSF drawn via a catheter during the i.v. infusion of very large amounts of radioiodinated CRF. It should be noted, however, that our method would not have detected concentrations in the CSF less than 1% of those in plasma. Previous work has shown that some peptides given peripherally exert central effects even when only 0.1% is shown to cross the blood-brain barrier.

In addition to causing ACTH secretion, we also noted that CRF caused dose-dependent growth hormone (GH) and prolactin secretory responses. These responses are of particular interest because these hormones are known to be secreted physiologically during stress along with ACTH. In order to determine a possible mechanism for CRF induced prolactin and GH secretion, we administered several pharmacological blocking agents to intact animals prior to and during their CRF administration procedures. We noted that naloxone significantly inhibits both

GH and prolactin responses, suggesting that beta-endorphin, which is secreted in response to CRF, may mediate the stimulation of these hormones. An alpha-adrenergic blocker inhibited only the GH response. Alpha-adrenergic blockade is also known to inhibit GH responses during hypoglycemic stress. We postulate that alpha-adrenergic blockade inhibited the GH-RH neuron from secreting GH-RH. Surprisingly, CRF was able to override dopamine blockade of prolactin secretion, while cyproheptadine, a serotonin antagonist, inhibited the GH response to CRF. None of the agents given influenced the HPA axis, suggesting that the CRF-induced ACTH response is mediated directly by a CRF receptor mechanism. That serotonin blockade failed to inhibit CRF-induced ACTH secretion is of interest in light of studies which show that cyproheptadine has been shown to be capable of reducing ACTH secretion in patients with Cushing's disease and possibly depression. Thus, the locus of cyproheptadine-induced inhibition of HPA function in these conditions would appear to be a central one, perhaps at the locus of the CRF neuron, rather than at the pituitary level.

To test the effects of ICV CRF administration in the intact primate, we administered CRF via an Omay-type indwelling catheter and reservoir in collaboration with Dr. E. Oldfield. Highly significant ACTH and plasma cortisol responses were seen following ICV CRF but not placebo at doses one-tenth of those required to achieve reliable responses after peripheral CRF administration. In the doses given, it is highly unlikely that ICV CRF would diffuse to the peripheral circulation in sufficient quantities to stimulate ACTH and cortisol secretion. Rather, it would appear more likely that CRF diffused from the site of injection (lateral ventricle) to the median eminence for transport down the hypophyseal portal system. This study suggests that the CSF may be a physiologically relevant pathway which conveys CRF to some of its sites of action, including possibly, the median eminence. Centrally-administered CRF also increased CSF ACTH concentrations. In contrast, peripherally administered CRF had no effect on CSF ACTH concentrations. These preliminary findings support the idea that CRF cannot cross the blood-brain barrier, and that there may be a group of ACTH secreting neurons which respond to central CRF and secrete into the CSF.

Development of Clinically Applicable CRF Stimulation Tests in Man

We initiated studies of CRF in normal volunteers with minute doses of i.v. CRF given as a bolus (.001 ug/kg) and gradually worked up to the dose of 1 ug/kg which best approximated the center of the dose response curve in primates. We elected to perform all these studies in the evening when the HPA axis is normally dormant. The lowest effective dose of ACTH secretion in humans was 0.1 ug/kg but patients tolerated the 1 ug/kg dose well. Parenthetically, when the 1 ug/kg dose was given to volunteers at 9 AM, no consistent neuroendocrine responses were noted though robust responses were seen when this dose was given in the evening. To date, in over 150 bolus injection studies using the 1 ug/kg dose in controls, patients with various diseases of the HPA axis, and in subjects with neuropsychiatric disorder, none has shown significant changes in pulse or blood pressure or displayed any serious side effects. None of the subjects who received CRF noted any discernible change in psychological state. Cognition was not formally tested. Fourteen of our 150 subjects reported facial and upper body flushing for 1-7 minutes after administration of the hormone, but this was not associated with subjective distress.

The plasma ACTH and cortisol responses to the 1 ug/kg dose in controls were prolonged and often had a biphasic pattern with a trough present at about 90 to 120 minutes after CRF administration. We postulate that the early peak represents release of stored ACTH, whereas the second represents induction of both synthesis and release. No growth hormone, prolactin, plasma arginine vasopressin, LH, FSH, insulin, or plasma glucose changes were seen at this dose. As part of this routine CRF bolus injection procedure, CRF itself was measured in 12 volunteers at frequent intervals after administration. As with the subhuman primate, CRF showed a biexponential disappearance curve with a short half-life of 11 ± 2 minutes and a long half-life of 73 ± 8 minutes. These values are longer than for any other hypothalamic releasing factor and probably account for the prolonged action of bolus CRF administration on ACTH and cortisol secretion. The volume of distribution was close to that of the blood volume. Extrapolating these data to the lowest peripheral effective dose in man, which is 0.1 mg/kg, we estimate that the minimal CRF concentration in plasma would be about 1 ng/ml required for biological activity. Since the cross reactivity of our antisera for human or primate CRF is unknown, we have not been able to draw conclusions about endogenous circulating CRF in primates or man.

In order to determine the effects of continuous infusion of CRF in man, we gave six volunteers CRF at a constant rate of 0.25 to 0.5 ug/kg/hr. Plasma immunoreactive CRF reached a steady state in 3-4 hours (about 5 ng/ml) and a minimum effective concentration (about 1 ng/ml) at 30 minutes. Plasma ACTH concentrations reached a peak at about 60 minutes, fell to an apparent nadir at 90 minutes, and rose to a plateau at 4 hours. Plasma cortisol rose initially to levels of about 40-50% above baseline and remained stable at this level for the entire infusion. These findings are different from those we obtained with continuous ACTH infusion (0.5 mg/kg/hr, $n = 12$) where cortisol levels rose steadily over 8 hours, and from the normal cortisol secretion pattern observed in 11 normal volunteers. These data suggest that in humans, continuous stimulation with CRF in vivo does not desensitize or hypersensitize the corticotroph. The fact that only a modest elevation in cortisol occurs with the CRF administration given continuously, in contrast to the profound elevation seen with ACTH administration, suggests that cortisol negative feedback serves as a restraint on continuous CRF excess. The pattern of cortisol hypersecretion seen during continuous CRF administration in man is analogous to the kind of cortisol hypersecretion seen in depression (see below), and is compatible with the hypothesis that depression represents a situation in which there is a chronic hypersecretion of CRF in the setting of intact feedback regulation. These findings also offer evidence against the hypothesis that Cushing's disease may result from increased and continuous hypothalamic secretion of CRF.

We have studied the neuroendocrine response to CRF in approximately 40 psychiatric patients with primary affective disorder, anorexia nervosa, and bulimia. We note that in a group of 12 drug-free patients with bipolar affective illness, there was a significant GH response, as measured by the net area under the curve, in contrast to controls, who showed no significant growth hormone response to CRF. In addition, the overall growth hormone net area in the affectively ill subjects was significantly greater than that seen in the controls. This finding may be of interest in light of our data that alpha-adrenergic blockade abolishes the GH response to CRF in primates. Although it is tempting to speculate that this response may constitute a marker

for bipolar illness, we have also observed an exceedingly robust growth hormone response to CRF in two drug-free, chronically underweight patients with anorexia nervosa. The ACTH responses to CRF were significantly lower in the four drug-free depressed patients compared to the controls. This preliminary data is compatible with the idea that there is intact feedback regulation of cortisol on ACTH release at the pituitary level. This pattern of blunted ACTH responses to exogenous CRF in depression, in association with our data of modest elevation of ACTH secretion during chronic CRF infusion in normals, suggests that the pathophysiological defect in depression with respect to HPA function may be a chronic increase in CRF secretion in subjects with fully intact feedback regulation at the pituitary level. Recovered and manic subjects with primary affective disorder showed normal ACTH responses although the responses tended to be highest in the manics. The cortisol responses to the CRF in bipolar affective illness showed the same pattern, i.e., higher responses in manics, normal responses in controls, and lower responses in depressed patients but none of these reached statistical significance, suggesting that the adrenal reacts much less sensitively than the pituitary and may not be nearly as good a marker for the functional activity of hypothalamic-pituitary function as is ACTH secretion. This concept is strengthened by the demonstration that above a certain ceiling of ACTH administration, the plasma cortisol response becomes maximal, so that there will be a rather fixed adrenal cortisol response to varying doses of ACTH above a threshold level.

We have preliminary data of pituitary responses to CRF administration in patients with eating disorders. Three chronically underweight anorexics showed exceedingly large ACTH responses to CRF (> 75 pg/ml) despite the fact that like the depressed subjects, these individuals were hypercortisolemic. Five bulimic subjects showed entirely normal ACTH responses. Since anorexics and bulimics, like patients with depression, often failed to suppress with dexamethasone administration, it will be of interest to see if CRF testing more sensitively distinguishes these entities with respect to hypothalamic-pituitary-adrenal responsiveness.

In collaborations with Drs. L. Loriaux and G. Chrousos, CRF was also given to subjects with significant disease of the HPA axis such as Cushing's disease and primary, secondary and tertiary adrenal insufficiency. Of considerable interest is the fact that patients with untreated Cushing's disease show a consistent rise in plasma ACTH and cortisol which is equal to or greater than the magnitude of the rise seen in subjects who are normal. This rise in ACTH seen in the Cushing's disease patient is significantly greater than that seen in patients with depression and suggests that the CRF stimulation tests may be a clinically meaningful way of distinguishing the two entities. In addition, patients with Cushing's disease failed to show the growth hormone elevation characteristic of patients with depression, thus constituting an additional marker obtained during CRF stimulation which may distinguish the two diseases. In contrast to patients with Cushing's disease, patients with ectopic ACTH syndrome failed to respond with an ACTH or cortisol response, indicating that the CRF test may also be a clinically useful tool in distinguishing pituitary from ectopic Cushing's disease. In patients with Cushing's disease studied after transphenoidal removal of an ACTH-secreting pituitary adenoma, there was normalization of the neuroendocrine responses, suggesting that the CRF test may predict successful surgical outcome. This normalization of ACTH responsiveness

soon after surgery suggests that adrenal insufficiency following successful adenectomy for Cushing's disease reflects residual suppression of the CRF neuron after prolonged hypersecretion of ACTH and cortisol rather than suppression at the pituitary level.

As expected, subjects with primary adrenal insufficiency show a marked ACTH response in association with undetectable cortisol secretion following CRF administration; moreover, patients with secondary adrenal insufficiency due to pituitary lesions had diminished ACTH and cortisol responses. CRF testing was able to separate patients with secondary adrenal insufficiency into two groups. Those patients with hypothalamic lesions had delayed, augmented ACTH responses, in contrast to the diminished ACTH responses seen in pituitary lesions. This pattern, although not identical to that seen in anorexia nervosa, is analogous to it, suggesting that anorexic patients may have hypothalamic CRF deficiency, in contrast to the depressed patients who have an excess of hypothalamic secretion of CRF. The hypercortisolemia seen in anorexia could reflect markedly decreased metabolism of cortisol and some degree of glucocorticoid receptor resistance and be present even in the setting of relative hypothalamic CRF deficiency. The finding of a delayed augmented ACTH response in hypothalamic disease suggests that the corticotroph cells in these patients retain their capacity to respond to CRF despite the fact that they have been under or unstimulated for a long time. Hence, the CRF test may be a way of detecting endogenous CRF deficiency.

Other Dynamic Tests of Function in Man:

In addition to our studies of HPA function using CRF as a probe, we have also devised other tests for examining the functional activity of this axis. An additional stimulation paradigm utilized for this purpose is that of continuous i.v. infusion of procaine, in collaboration with Drs. C. Kellner and R. Post. We note that i.v. procaine infusion causes an immediate and pronounced increase in ACTH secretion as well as a synchronous secretion of beta-endorphin. Since the locus of the procaine stimulation is most likely the hypothalamic level, the capacity to stimulate hypothalamic CRF secretion with a benign test such as this one represents a major advance over the only other available test for hypothalamic CRF reserve, that is, the insulin tolerance test. Thus, the procaine stimulation test could potentially replace the insulin tolerance test as the standard test in clinical medicine for assessing the functional integrity of the hypothalamic neuron secreting CRF. Speculatively, since CRF has been shown to induce limbic seizures, procaine's effects on limbic electrical activity could be partially mediated by this peptide.

We have also evaluated HPA function by assessing the effects of opiate administration on ACTH and cortisol secretion. We previously noted that acute i.v. methadone administration significantly depresses plasma cortisol secretion in depressed patients, and have suggested that methadone administration could be utilized as a test of the set point for feedback inhibition of the opiates on HPA activity. In a more recent study in collaboration with Dr. George Chrousos, we have also administered parenteral morphine to 11 normal volunteers, 16 patients with Cushing's disease, and 4 patients with depression. Each subject was studied in a double-blind, placebo crossover design. Blood was drawn every 30 min. for 28 hours starting at 0800. Morphine sulfate, 10 mg, or placebo was given subcutaneously every 4 hours between 20:00 and 12:00 the following day.

In normals, morphine sulfate significantly suppressed the circadian cortisol rise, the integrated cortisol levels, and the frequency of secretory episodes. A phase delay of 2 hours was noted in the cortisol surge in the morphine sulfate treated subjects. Patients with depression showed similar responses but those with Cushing's did not respond to morphine sulfate. We conclude, therefore, that morphine, and by inference, beta-endorphin, suppresses circadian and stress-induced ACTH secretion, suggesting that endogenous opioid peptides participate in the physiologic feedback of the HPA axis. The locus of suppression is unknown but may be in the hypothalamus, where the frequency of episodic secretion is regulated and where opiate receptor concentration is high. In addition, the observed phase delay suggests that opiates affect the circadian oscillators. The decreased cortisol levels and the phase delay of the circadian cortisol surge during opiate treatment are the mirror image of what is seen in depression, in which cortisol levels are elevated and the circadian rise is advanced. This suggests the possibility of a state of relative endorphin deficiency in depressed patients. In addition, one further aspect of possible significance in this study is that there seems to be a differential response to morphine in the depressed patients in Cushing's, suggesting that this may be yet another test capable of distinguishing these two entities on the basis of neuroendocrine responsivity.

An additional study of HPA function in primary affective disorder has been the assessment of 24 hour urinary free cortisol secretion in these patients compared to controls. A large number of subjects (240) participated in the study and at least three urinary free cortisol determinations were assessed in each individual. Our findings show that using the interclass correlation coefficient as a guide, three urinary free cortisol determinations are necessary to give a reliable estimate of corticosteroid secretion in a given individual, and that fewer determinations, as is characteristic of almost every other study, do not represent a reliable index of this parameter given the day-to-day variability in various patient subgroups and controls. We note, as expected, that urinary cortisol excretion is significantly increased in unipolar and bipolar depressed patients compared to controls; the magnitude of the increase is similar in each subgroup and runs roughly 30-35% greater in depressed patients compared to controls, a figure which is analogous to the degree of ACTH and cortisol elevation seen in normals when they are given continuous CRF infusions. We note that urinary free cortisol excretion is significantly lower in manic patients than depressed patients though the manics did not differ from controls.

Other Studies of HPA Function in Man:

An additional study of the hypothalamic-pituitary-adrenal axis in patients with primary affective disorder was headed by Dr. Charles Kellner, who examined the relationship between urinary free cortisol secretion and cerebroventricular size in ten depressed patients. Dr. Kellner undertook this study in light of previous reports that patients with endogenous hypercortisolemia, particularly those with Cushing's disease, show ventricular atrophy which is reversible when the hypercortisolism is corrected. Dr. Kellner noted a significant positive correlation between the magnitude of urinary free cortisol excretion and the ventricular brain ratio.

Given our significant interest in HPA functional activity in patients with

primary affective disorder, we have obtained an IND to administer a compound which actually alters the dynamic regulation of this axis in patients with depression. Specifically, we are administering metyrapone, a cortisol synthesis inhibitor, to patients with depression. The rationale for this study is that by interfering with cortisol secretion, we hope to significantly increase pituitary beta-endorphin secretion in hopes of ameliorating the affective component of the depressive syndrome and of increasing the levels of endogenous opiates in the CNS. Previous anecdotal reports have suggested that metyrapone, when given to hypercortisolemic patients thought to have Cushing's disease, who were also depressed, significantly ameliorated the depression and that this depression did not subsequently recur, suggesting that metyrapone actually ameliorated the depressive symptomatology in these patients. To date we have administered two courses of metyrapone to one bipolar patient with affective disturbance. In the first instance, the patient had clinical remission of depression during metyrapone administration in association with increased beta-endorphin secretion. It was unclear, however, whether this clinical remission was secondary to the metyrapone or was simply a spontaneous switch out of depression, although the switch out of depression clearly occurred on the 10th day of metyrapone administration. In a second trial the patient did not have a profound amelioration of her depression although it was the consensus of the ward staff that the depression, though palpable and continuously present during the metyrapone administration, was of a lesser degree than any of the previous depressions which had been observed in the patient. Parenthetically, three days after cessation of the metyrapone and following institution of lithium carbonate treatment, the patient experienced an abrupt switch into hypomania.

(2) Arginine Vasopressin (AVP)

We are continuing comprehensive investigation of arginine vasopressin function, in collaboration with Dr. Gary Robertson, concentrating on studies of CSF AVP, the plasma AVP response to hypertonic saline, and the cognitive and behavioral responses to vasopressin analog administration. We have recently initiated a series of studies to examine non-osmolar stimuli to plasma secretion of AVP to directly evaluate neurohypophyseal function. We have studied AVP function in a variety of neuropsychiatric illnesses including anorexia nervosa, primary affective disorder, and schizophrenia.

We have previously noted significant abnormalities of AVP secretion into the CSF and plasma of patients with anorexia nervosa and patients with affective illness. We have also noted significant effects of lithium and carbamazepine on the osmoregulation of plasma AVP. In studies of osmoregulation in drug-free psychotic schizophrenic subjects we have shown that the osmoregulation of AVP is entirely normal. This was noted in 18 consecutive subjects studied with hypertonic saline infusion. Thus, abnormalities in AVP secretion either in threshold or sensitivity do not appear to be common in patients with schizophrenia and the reported cases of water intoxication in these subjects would not appear to be reflections of systematic abnormal AVP secretion defects but rather idiosyncratic responses. It should be noted, moreover, that the pattern of AVP secretion to osmotic stimulation in schizophrenia is in contrast to that seen in bipolar depression so that the pattern of responses could be helpful in distinguishing the two entities. We note that CSF AVP is subtly but significantly lower in a group of 52 schizophrenic patients compared to 39

normal volunteers. The significance of these findings with respect to the altered cognition in schizophrenia is unclear.

Since we noted in our depressed patients that there is a reduced sensitivity and/or delayed threshold of AVP secretion and since indirect tests suggest that hypercortisolemia could delay the threshold for vasopressin secretion, we examined the effects of an antigluocorticoid synthetic steroid on a variety of pituitary responses including that of AVP. This compound, RU486, significantly elevated circulating levels of AVP, suggesting indeed that corticosteroids tonically influence AVP secretion and that hypercortisolemia could contribute to the responses noted in our depressed subjects. We also noted that RU486 inhibited prolactin secretion induced by an estrogen-progesterone synergy, and in addition to elevating the vasopressin levels, also elevated ACTH and cortisol secretion, as expected. In contrast, plasma FSH-LH, growth hormone and TSH levels were unaffected. Further clinical tests of RU486 are in progress as a probe of HPA function in patients with depression and Cushing's disease, as well as on its potential therapeutic effects in Cushing's disease and, in light of its anti-progesterone effects, as a possible simple and safe contraceptive.

(3) Oxytocin (OT)

We have also measured the level of oxytocin in CSF and note that oxytocin, which is a structural analog of AVP, is routinely present in the CSF of both male and female subjects. There were no significant differences in the level of CSF OT between men and women, a result which is compatible with findings in experimental animals that estrogen does not influence the secretion of oxytocin into the CSF, in contrast to its effects on the levels of OT secreted into plasma. In psychiatric patients, we have extended our data which continues to support the previous finding that CSF OT is significantly reduced in drug-free manic patients, a finding which is the converse of the pattern seen with respect to AVP secretion. This finding of increased AVP and reduced OT in the CSF of manics is of interest in light of the reciprocal effects which AVP and OT have been reported to exert on cognition, REM sleep, hippocampal theta rhythms and ACTH secretion. We also note that the level of OT is the reciprocal of that AVP in anorexia nervosa; that is, whereas AVP levels are high in the CSF of underweight anorexics, OT levels are reduced.

(4) CSF Somatostatin

We are continuing to investigate the levels of somatostatin in the CSF of patients with primary affective disorder in collaboration with Drs. David Rubinow, Robert Post and Seymour Reichlin. Dr. Rubinow has previously published that somatostatin is significantly lower in a large group of drug-free unipolar and bipolar depressed patients compared with controls, and that psychoactive agents such as carbamazepine and zimelidine significantly influence the levels of somatostatin in CSF. The significance of these findings is currently under investigation.

(5) Growth Hormone Releasing Factor

In collaboration with Drs. George Merriam, George Chrousos, and Lynn Loriaux, we have begun evaluating the pituitary somatotrophs with bolus infusion of growth hormone releasing factor, a recently sequenced peptide with potent GH releasing properties. In a pilot study in controls, this substance has been shown to be completely safe and to cause an extremely robust dose-related

increase in growth hormone secretion. We have chosen the dose that is at the center of the dose response curve, and have initiated studies in psychiatric subjects with known abnormalities in growth hormone secretion, namely, patients with primary affective disorder and anorexia nervosa.

We continue to apply dynamic tests of monoaminergically regulated hypothalamic-pituitary systems and are evaluating our data utilizing continuous dopamine infusions, neuroendocrine effects on prolactin, growth hormone and LH secretion. Preliminary data suggest that the growth hormone response to dopamine infusion and the LH decrement during this paradigm is exaggerated in drug-free schizophrenic patients compared to controls.

Significance to Biomedical Research and to the Program of the Institute

The clinical work of the Unit on Neuroendocrinology focuses on studies of central peptide function in three populations of subjects: 1) patients with major affective illness, particularly manic-depressive illness and anorexia nervosa; 2) patients with neuroendocrine abnormalities, particularly Cushing's disease; and 3) normal controls. Some observations which have been made in these subjects of significance to biomedical research include 1) establishment of a safe and methodologically sound CRF stimulation paradigm; 2) elucidation of the pharmacokinetics of CRF in man; 3) development of two stimulation paradigms (i.e., CRF stimulation and morphine stimulation) which seem capable of providing a differential diagnosis between depression and Cushing's disease; 4) development of a potential diagnostic marker for depression (i.e., blunted ACTH responses to CRF); 5) demonstration of abnormal growth hormone responses to CRF in drug-free patients with primary affective disorder; 6) development of a procaine infusion paradigm which may potentially replace the insulin tolerance test in clinical medicine; 7) performance of major studies of AVP secretion into the CSF and plasma compartments of patients with anorexia nervosa, affective illness and schizophrenia. In anorexia nervosa we have described a new syndrome of abnormal osmoregulation invariably associated with abnormal AVP secretion into the CSF compartment. In affective illness we have shown subtle defects in plasma and CSF AVP secretion. Moreover, we have also further elucidated the pharmacologic control of osmoreceptor function in man.

In three clinical studies in intact and stalk-sectioned primates, we have elucidated the biological effects and pharmacokinetics of peripheral CRF administration and documented the effects of monoaminergic neurotransmitters on CRF stress hormone release. We have also demonstrated that CRF is actively cleared from the CSF, and that ICV CRF has biological effects, suggesting that the CSF is a physiologically relevant pathway mediating CRF effects on CNS function. We have also developed a model for studying CRF secretion into the CSF in the sheep since our antisera to ovine CRF cross reacts and measures CRF in this compartment, and are actively pursuing studies of the neuroregulation of CRF secretion into this compartment.

Proposed Course of the Project

We shall continue to study hypothalamic-pituitary-adrenal regulation in primary affective disorder, anorexia nervosa, and Cushing's disease. Areas of major interest include further elaboration of the specific locus and defect in HPA regulation in these entities, further elucidation of the clinical utility of CRF testing, and refinement of other dynamic tests of HPA function, including stimu-

lation by glucocorticoid receptor blockade and inhibition by opiate agonism. In addition we have commenced measurement of ACTH, beta-endorphin and other fragments of proopiomelanocortin in our clinical populations with assays developed in our laboratories. We shall also actively pursue the study of neurohypophyseal function in these clinical populations. A major emphasis will be on the study of the possible role that AVP plays in appetite regulation and cognition in anorexia nervosa. A major new arena of active investigation is the primate laboratory where we will continue to study biological and pharmacokinetic properties of CRF in the plasma and CSF compartments. In other preclinical studies, we have been working for the past year on refining an animal model of depression which responds to treatment with traditional antidepressant interventions so that we can extend our studies now being conducted in clinical populations. We have applied a technique for continuous sampling of ventricular fluid in the sheep, where we can measure CRF in low concentrations, utilizing an antibody raised against synthetic ovine CRF. With this model we are actively exploring the regulation of CRF secretion into the CSF. We plan to expand our radioimmunoassay laboratory to include not only the potential for the development of a variety of new peptide assays, but also with the capacity to generate samples on a non-collaborative basis with other investigators in the NIMH.

Publications

- Gold, P.W., Post, R.M., Weingartner, H., and Goodwin, F.K.: Central peptide function in affective illness. Arginine vasopressin as a model system. In de Groot, L. (Ed.): Advances in Biological Psychiatry, Vol. 7. Basel, S. Karger, 1982, pp. 126-152.
- Schulte, H.M., Chrousos, G.P., Oldfield, E.H., Gold, P.W., Cutler, G.B., and Loriaux, D.L.: The effects of corticotropin releasing factor on the anterior pituitary function of stalk-sectioned cynomolgus macaques: Dose response of cortisol secretion. J. Clin. Endocrinol. Metab. 55: 810-813, 1982.
- Schulte, H.M., Chrousos, G.P., Gold, P.W., Oldfield, E.H., Philips, J.M., Munson, P., Cutler, G.B., and Loriaux, D.L.: Metabolic clearance rate and plasma half-life of radioiodinated corticotropin releasing factor in a nonhuman primate. J. Clin. Endocrinol. Metab. 55: 1023-1026, 1982.
- Becker, L.E., Chrousos, G.P., and Gold, P.W.: Analogies between Cushing's Disease and depression. Hospital Psychiatry 5:32-35, 1983.
- Gold, P.W., Kay, W., Robertson, G.L., and Ebert, M.H.: Abnormalities in plasma and cerebrospinal fluid arginine vasopressin in patients with anorexia nervosa. N. Engl. J. Med. 308: 1117-1123, 1983.
- Gold, P.W., Robertson, G.L., Post, R.M., Kaye, W.H., Ballenger, J.C., Rubinow, D.R., and Goodwin, F.K.: The effect of lithium on the osmoregulation of arginine vasopressin secretion. J. Clin. Endocrinol. Metab. 56: 295-299, 1983.
- Kellner, C., Roy-Byrne, P., Rubinow, D.R., Gold, P.W., and Post, R.M.: Cerebral atrophy in torture victims. N. Engl. J. Med. 308:903-904, 1983.
- Kellner, C.H., Rubinow, D.R., Gold, P.W., and Post, R.M.: Relationship of cortisol hypersecretion to brain CT scan alterations in depressed patients. Psychiatry Res. 8: 191-197, 1983.
- Gold, P.W.: Neurohormones in psychiatry. In Flach, F. (Ed.): Directions in Psychiatry, Monograph #31. New York, Hatherleigh Press, 1983.
- Gold, P.W., Ballenger, J.C., Robertson, G.L., Goodwin, F.K., Rubinow, D.R., Kaye, W., and Post, R.M.: Carbamazepine diminishes the sensitivity of the plasma arginine vasopressin response to osmotic stimulation. J. Clin. Endocrinol. Metab. 1983, in press.
- Schulte, H.M., Chrousos, G.P., Gold, P.W., Booth, J.D., Oldfield, E.H., Cutler, G.B., and Loriaux, D.L.: Corticotropin releasing factor: Pharmacokinetics in man. J. Clin. Endocrinol. Metab., 1983, in press.
- Healy, D.L., Chrousos, G.P., Schulte, H.M., Williams, R.F., Gold, P.W., Baulieu, E.E., and Hodgen, G.D.: Pituitary and adrenal responses to the anti-progesterone and anti-glucocorticoid steroid RU486 in primates. J. Clin. Endocrinol. Metab., 1983, in press.

- Chrousos, G.P., Gold, P.W., Schulte, H.M., Cutler, G.B., and Loriaux, D.L.: Effects of opiates on the human hypothalamic-pituitary-adrenal axis: opiates suppress the corticotropin releasing factor neuron and cause phase delay of the circadian cortisol oscillator. Acta Endocrinol., Suppl., 1983, in press.
- Schulte, H.M., Chrousos, G.P., Gold, P.W., Oldfield, E.L., and Loriaux, D.L.: Corticotropin releasing factor: A common link between anterior pituitary and sympathetic responses in stress. Acta Endocrinol., Suppl., 1983, in press.
- Schulte, H.M., Chrousos, G.P., Oldfield, E.H., Gold, P.W., Booth, J.D., Cutler, G.B., and Loriaux, D.L.: Continuous corticotropin releasing factor administration in man and nonhuman primates. Acta Endocrinol., Suppl., 1983, in press.
- Chrousos, G.P., Schulte, H.M., Oldfield, E.H., Doppman, J.L., Gold, P.W., Cutler, G.B., and Loriaux, D.L.: The corticotropin releasing factor stimulation test: An aid in the diagnosis of Cushing's syndrome. Acta Endocrinol., Suppl., 1983, in press.
- Gold, P.W., Ballenger, J.C., Weingartner, H., Post, R.M., Robertson, G.L., and Goodwin, F.K.: Vasopressin studies in affective illness: Levels in CSF, response to hypertonic saline, and effects of chronic administration. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of Mood Disorders. Baltimore, Williams & Wilkins, 1983, in press.
- Rubinow, D.R., Post, R.M., Gold, P.W., Ballenger, J.C., and Goodwin, F.K.: Cortisol dysregulation in patients cycling between depression and mania: Serum, urinary, and catheter studies. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of Mood Disorders. Baltimore, Williams & Wilkins, 1983, in press.
- Rubinow, D.R., Gold, P.W., Post, R.M., and Ballenger, J.C.: Somatostatin in affective illness. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of Mood Disorders. Baltimore, Williams & Wilkins, 1983, in press.
- Gold, P.W., Rubinow, D.R., Post, R.M., Ballenger, J.C., Fisher, D.A., Goodwin, F.K., and Robertson, G.L.: Neurohypophyseal hormones in primary affective disorder and anorexia nervosa. Psychopharmacol. Bull., 1983, in press.
- Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C., and Reichlin, S.: Reduced cerebrospinal fluid somatostatin in unipolar and bipolar depression. Psychopharmacol. Bull., 1983, in press.
- Chrousos, G.P., Schulte, H., Cutler, G., Loriaux, D.L., Kellner, C., and Gold, P.W.: Corticotropin releasing hormone: Physiology and kinetics in primates and neuroendocrine responses in psychiatric patients and controls. Psychopharmacol. Bull., 1983, in press.
- Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C., Goodwin, F.K. and Reichlin, S.: Cerebrospinal fluid somatostatin in patients with primary affective disorder. Arch. Gen. Psychiatry, 40: 409-412, 1983

Gold, P.W., Ballenger, J.C., Robertson, G.L., Fisher, D.A., Goodwin, F.K., and Post, R.M.: Arginine vasopressin and oxytocin secretion in patients with primary affective disorder, anorexia nervosa, and schizophrenia. Prog. Neuropsychopharmacol. Biol. Psychiatry, 1983, in press.

Rubinow, D.R., Gold, P.W., Post, R.M., Ballenger, J.C., Goodwin, F.K., and Reichlin, S.: Somatostatin in cerebrospinal fluid: Levels, relationship to gradient, and impact of psychotropic agents. Prog. Neuropsychopharmacol. Biol. Psychiatry, 1983, in press.

Chrousos, G.P., Schulte, H.M., Kellner, C., Cutler, G.B., Post, R.M., Loriaux, D.L., and Gold, P.W.: Corticotropin releasing factor in primates and human subjects. Prog. Neuropsychopharmacol. Biol. Psychiatry, 1983, in press.

Gold, P.W.: The study of neurohormonal function in psychiatry. Prog. Neuropsychopharmacol. Biol. Psychiatry, 1983, in press.

Oldfield, E.H., Schulte, H.M., Chrousos, G.P., Gold, P.W., Cutler, G.B., and Loriaux, D.L. Corticotropin releasing factor stimulates ACTH secretion in Nelson's syndrome. J. Clin. Endocrinol. Metab., in press.

Chrousos, G.P., Schulte, H.M., Gold, P.W., Oldfield, E.H., Cutler, G.B., and Loriaux, D.L. Responses to corticotropin releasing factor in primary, secondary, and tertiary adrenal insufficiency. J. Clin. Endocrinol. Metab., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00124-06 BP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Mechanism of Action of Lithium in the Treatment of Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Agu Pert, Ph.D., Psychologist, Biological Psychiatry Branch, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Unit on Behavioral Pharmacology

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

0

PROFESSIONAL:

0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Work on this project has been temporarily postponed pending the establishment of an appropriate autoradiographic facility and the acquisition of autoradiographic skills by laboratory personnel.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00147-08 BP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral and Physiological Effects of Brain Peptides and Other Psychoactive Compounds		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Agu Pert, Ph.D., Psychologist, Biological Psychiatry Branch, NIMH		
COOPERATING UNITS (if any) Lab. of Cerebral Metabolism, NIMH Neurosciences Branch, NIMH Laboratory of Clinical Science, NIMH		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Unit on Behavioral Pharmacology		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Calcitonin receptors</u> were found in uniquely high concentrations in the <u>periaqueductal gray matter</u> (PAG) of the rat. Injections of calcitonin into this region produced profound analgesia. Iontophoretic applications of <u>neurotensin</u> to PAG neurons increased their firing rate. Injections of <u>neurotensin</u> into the PAG also increased the firing rate of <u>raphe magnus</u> neurons, indicating an activation of <u>descending inhibitory mechanisms</u>. Injections of <u>neurotensin</u> into the <u>substantia nigra</u> and <u>caudate nucleus</u> increased dopamine metabolites in these structures without increasing release. <u>Cholecystokinin</u> receptors were found to be localized on intrinsic non-dopaminergic cell bodies in the <u>striatum</u> and <u>substantia nigra</u>. </p> <p> Employing the new <u>in vivo</u> autoradiographic procedures, this was found to increase release of <u>endorphins</u> in the PAG, <u>hypothalamus</u>, <u>midline thalamic</u> structures and <u>reticular formation</u>. Employing <u>local cerebral glucose utilization</u> (LCGU) procedures, <u>rewarding brain stimulation</u> of the <u>ventral tegmental area</u> was found to increase glucose consumption in a highly circumscribed zone that extended both laterally and caudally through the <u>medial forebrain bundle</u>, extending via the <u>diagonal band of Broca</u> to the level of the <u>pre-optic area</u>. LCGU procedures also revealed that <u>nigral dopamine</u> neurons are essential for metabolic activation of the <u>entopeduncular</u> and <u>subthalamic nuclei</u> in <u>nigral stimulated rats</u>. <u>Opiate receptors</u>, with a <u>mu ligand selectivity pattern</u>, showed striking <u>laminar heterogeneity</u> in rat cortex and were densest in limbic cortical areas. </p>		

Project Description

Objectives

Analgesic Effects of Opiate and Non-opiate Neuropeptides

Opiate peptides as well as opiate alkaloids are known to induce their analgesic effects through the periaqueductal gray matter (PAG). For example, injections of opiates into the PAG, which is high in opiate receptors and opiate peptides, produce profound analgesia in the rat. Besides endorphins, the PAG also contains relatively high concentrations of other neuropeptides and neuropeptide receptors. Previously we have demonstrated that a number of neuropeptides, including neurotensin, VIP and bombesin, induce analgesia following injections into the PAG. More recently, calcitonin (CT) has been shown to evoke long-lasting analgesic effects that are not opiate-mediated following intraventricular or intrathecal applications in rodents and man. Little, however, is known regarding the specific sites of action of this peptide in eliciting analgesia. For this study, we have visualized the calcitonin receptor distribution in the rat midbrain and hindbrain with autoradiographic procedures using ^{125}I -salmon calcitonin and then evaluated the analgesic effects of both human (hCT) and salmon (sCT) calcitonin following direct injections into the PAG, a region of uniquely high CT binding.

We have previously shown that injections of neurotensin into the PAG elicit profound analgesia which is attenuated by radiofrequency lesions of the raphe magnus region. In this series of studies we evaluated the electrophysiological effects of iontophoretic applications of neurotensin on PAG neurons. We also examined the effects of direct applications of neurotensin into the PAG on raphe magnus neuronal firing.

Modulation of Dopaminergic Activity by Neurotensin

There is considerable evidence of important interactive effects between neurotensin and dopamine. However, it is still not clear how and where in the brain neurotensin exerts its effects on dopamine neurotransmission. In this series of studies, neurotensin (1.0 or 10.0 nmoles in 1 μl saline) was administered unilaterally into the substantia nigra or caudate nucleus of male Sprague-Dawley rats. Rotational behavior was automatically recorded for 30-45 min. following neurotensin administration. The rats were then immediately decapitated and the ipsilateral and contralateral substantia nigra and caudate nucleus were micro-dissected. The fresh frozen brain samples were assayed within 24 hrs. by an HPLC procedure for the simultaneous determination of L-DOPA, dopamine, serotonin and their metabolites.

Relationship of Cholecystokinin and Neurotensin Receptors to the Mesolimbic and Nigrostriatal Dopamine Systems.

It has been shown that cholecystokinin (CCK) coexists with dopamine (DA) in a certain population of DA-containing cells. Furthermore, it has also been found that CCK exerts effects on DA neurotransmission. The purpose of this series of studies was to ascertain the precise localization of CCK receptors in relation to mesolimbic and nigrostriatal DA neurons.

Nigrostriatal dopaminergic projections were lesioned unilaterally by intranigral injections of 6-hydroxydopamine (6-OHDA) (9 ug). Mesolimbic dopaminergic projections were lesioned unilaterally by injection of 6-OHDA (7.5 ug) into the ventral tegmental area. Unilateral lesions of the medial forebrain bundle (MFB) were also made by injections of 6-OHDA into this fiber bundle at the level of the lateral hypothalamus. Intrinsic neurons in the striatum and substantia nigra (SN) were destroyed by unilateral injections of ibotenic acid (10 ug). One month later the animals were killed and their brains removed. Serial 25 um sections were taken through the forebrain region encompassing the nucleus accumbens and caudate nucleus and the midbrain region encompassing the substantia nigra and ventral tegmental areas. These sections were then exposed to [³H]pentagastrin or Na[¹²⁵I] des-aminotyrosyl] CCK-33 and processed for autoradiography using a tritium-sensitive film.

Visualization of Changes in Opiate Receptor Occupancy due to Behavioral Manipulations

A method of *in vivo* autoradiography was utilized which allows the indirect visualization of functional opiate peptide release, based on the assumption that prior receptor occupation will exclude the binding of an exogenously applied, tritium-labeled, opiate ligand. Coupled with tritium-sensitive film autoradiography, it allows the mapping of relative levels of behavior-specific receptor occupancy throughout the whole brain.

We tested two types of stress manipulations which are known to release endorphins; forced swims in cold water (40°C for 3.5 min.) and prolonged intermittent footshock (1 ma. for 20 min., 1 sec. on, 5 sec. off). Both of these have been shown to increase nociceptive thresholds in a naloxone-reversible manner. Following the stress, the high affinity opiate antagonist, ³H-diprenorphine was injected via an indwelling jugular catheter (50 uCi/kg, 0.002 mg/kg). After 20 minutes (to allow washout from non-specific sites), the rats were decapitated and the brains frozen intact for slicing. Unstressed control rats were prepared identically and matched to stressed rats on the basis of cerebellar and liver (non-specific) binding levels. All comparisons made were between anatomically congruent sections from these matched pairs, exposed on the same sheet of film.

Metabolic Mapping of Nigral Connections and Circuitry Underlying Rewarding Brain Stimulation with the 2-[¹⁴C] Deoxyglucose Method

The quantitative 2-[¹⁴C] deoxyglucose method was used to determine local cerebral glucose utilization in unrestrained rats responding in an operant situation for rewarding electrical stimulation to the ventral tegmental area and in similarly implanted inactive controls. The 2-DG method was also employed to assess cerebral glucose utilization during unilateral electrical stimulation of the substantia nigra. In this study we assessed to what extent nigral dopamine neurons contribute to the metabolic activation of basal ganglion structures. Six groups of rats were utilized: 1) sham stimulation, 2) unilateral nigral stimulation, 3) nigral stimulation in animals pretreated with haloperidol (0.1 mg/kg i.v., 20 minutes prior to stimulation), 4) unilateral lesion of the medial forebrain bundle with 6-OHDA, 5) 6-OHDA lesion + nigral stimulation, 6) 6-OHDA lesion + nigral stimulation + haloperidol.

Effects of Nicotine on Nigrostriatal Dopaminergic Activity

There is suggestion that nicotine may enhance DA activity. The rotational model was used to evaluate whether acute and chronic nicotine enhanced activity in the dopaminergic nigrostriatal pathways. Rats were lesioned unilaterally in the substantia nigra with 6-OHDA. Two weeks later they were injected with either 0.1, 0.2, 0.4 or 0.8 mg/kg of nicotine tartrate and placed in automated rotometers. Following the determination of the dose-response function, the animals were divided into two groups. One group was injected daily for 2 weeks with nicotine and the other group was injected with saline. At the end of this period, both groups were tested in the rotometers following an injection of nicotine.

Autoradiographic Distribution of Opiate Receptors in Rat Cortex

The differential distribution of [^3H] naloxone-labeled and [^3H] D-ala-D-Leu-enkephalin-labeled opiate receptors in rat cerebral cortex were localized autoradiographically and quantified by grain counting and computerized densitometry. In addition, receptor distributions were compared to terminal patterns of thalamocortical projections labeled by axoplasmic transport of [^3H] amino acids.

Major Findings:

The autoradiographic analysis of calcitonin receptor distribution in rat brain revealed exceptionally heavy binding of sCT in the ventral and ventrolateral segments of the PAG extending along the entire rostral-caudal axis. The mesencephalic reticular formation and the nucleus tractus spinalis nervi trigemini were also relatively high in sCT binding at this level of the brain.

After intracerebral injections, sCT was found to induce a dose-dependent increase in hot-plate latencies but appeared to have little effect in the tail flick test. At all doses tested, the effect was significant between 15 and 30 min., reaching a peak at 60 min. and lasting throughout the duration of testing. Human CT was much less effective than salmon CT in eliciting an analgesic effect and was also less potent in displacing ^{125}I -sCT bound to slide-mounted sections of rat PAG. Autoradiographic and histological analyses revealed that in rats in which CT elicited a significant analgesic effect, the injection had been made into the area of the PAG containing high CT binding. Injections which were less effective or ineffective had been made outside of this region. These findings suggest that the ventral aspect of the PAG may be an important site of action of CT or a CT-like peptide in eliciting analgesia. Neurotensin analgesia induced by injections of this neuropeptide into the PAG were accompanied by significant electrophysiological effects. NT injections into the PAG were found to increase the firing rate of 20 out of the 35 cells recorded from the nucleus raphe magnus region. The time of onset for the excitatory response was highly variable. The mean latency to onset was 15 seconds with a range from 7 to 65 seconds. Of the 50 cells in the PAG, 32 cells responded to NT by excitation. The onset of response to NT application was variable and ranged from 10 to 40 seconds. The activity of the cells remained elevated for as long as 2 min. after the NT application had terminated. These findings indicate that the excitatory effects of NT in the PAG are translated to an excitatory action in the area of the raphe magnus. Activation of these cells in turn probably increases the influence of descending inhibitory systems to nociceptive dorsal horn cells in the spinal cord, attenuating the transmission of pain information through this relay.

While the administration of neurotensin failed to elicit consistent rotational behavior, significant dose-dependent increases in the acidic metabolites of dopamine ipsilateral to the site of injection were found. Nigral administration of neurotensin (10.0 nmoles) produced 30-80% increases in the nigral contents of DOPAC and HVA with concomitant 100-190% increases in these metabolites in the ipsilateral caudate nucleus. The striatal content of dopamine was increased about 60% ipsilaterally after injections of the high dose of neurotensin into either the substantia nigra or caudate nucleus. Neurotensin increased the NSD-1015-induced accumulation of L-DOPA in the caudate nucleus from 1.8 ± 0.2 to 2.9 ± 0.3 ng/ml. Similar effects on dopamine and dopamine metabolism were observed in the nucleus accumbens following intranigral injections. Serotonin and 5-HIAA contents in the nigrostriatal system were not altered significantly by treatment with neurotensin. These behavioral and neurochemical observations indicate that neurotensin increases the synthesis of dopamine and the intraneuronal metabolism by MOA, whereas it decreases the neuronal activity or shuts off the release of dopamine. This notion is consistent with the results of the following experiment with d-amphetamine. Unilateral intra-administration of neurotensin resulted in no significant rotational behavior. However, when d-amphetamine (2.5 mg/kg, i.p.) was administered 15 min. later, significant turning contralateral to the injection site was observed in 10 of 14 rats. AMPT pretreatment significantly attenuated this amphetamine-induced rotational behavior. In conclusion, neurotensin could turn off the dopaminergic activity in the nigrostriatal system in a manner similar to axonal transection or treatment with gamma-butyrolactone.

Comparative autoradiographic distribution of cholecystokinin (CCK) receptors in rat brain using [^3H]pentagastrin or $\text{Na}^+[\text{}^{125}\text{I}]$ desaminotyrosyl CCK-33 showed high densities of CCK receptors in the nucleus accumbens, especially its medial part, moderate levels in the striatum and substantia nigra, and very low levels in the ventral tegmental area. Since these brain areas contain dopamine terminals and cell bodies, respectively, it was of interest to ascertain the precise localization of CCK receptors in relation to mesolimbic and nigrostriatal dopamine neurons.

6-OHDA lesions of the SN failed to alter CCK binding in either the ipsilateral striatum or SN. A-10 lesions were also ineffective in this regard. MFB lesions, which produce retrograde degeneration of DA neurons in the zona compacta of the SN, were also ineffective in modifying CCK binding in the SN. Injections of ibotenic acid in the striatum and SN, on the other hand, effectively decreased CCK binding at the site of injection.

Altogether, these results suggest that CCK receptors are localized on intrinsic non-dopaminergic cell bodies in the striatum and SN and that DA terminals in the striatum and nucleus accumbens are devoid of CCK receptors.

Stress was found to cause greatly decreased opiate receptor binding to most caudal subcortical structures, while cortex, hippocampus, and more rostral structures were largely unchanged. Greatest decreases (up to 60%) were seen in brain areas such as periaqueductal gray, reticular formation, and midline-intralaminar nuclei of thalamus, reflecting opiate peptide release in areas which receive and modulate incoming pain information. Lesser decreases in binding were seen in hypothalamus and limbic areas such as amygdala, the bed nucleus, and preoptic area. Motor areas of thalamus and the basal ganglia showed still smaller effects. In

the footshock group, decreases were similar but less extreme. Periaqueductal gray, reticular formation, and the midline thalamic areas showed decreases of up to 30%, while limbic changes were slightly smaller. No changes unique to the footshock treatment were seen, suggesting similar opiate responses to painful stress of differing modalities.

This in vivo autoradiographic technique holds great promise, both for the study of other behaviorally-induced changes in patterns of opiate release, and for adaptation to other neurotransmitter systems.

Results revealed activation of discrete neuronal pathways and particular projection sites as assessed by increases in rates of local cerebral glucose utilization (LCGU). Comparison between ipsilateral (stimulated) and contralateral sites showed marked activation, highly circumscribed in the VTA, that continued rostrally within a rather compact zone of activity, congruent with the well characterized mesotelencephalic DA projections up through the MFB, and extending through the diagonal band of Broca to the level of the pre-optic area. In the terminal projection fields, increases in LCGU were noted only in the central amygdaloid nucleus and medial prefrontal cortex. Caudal to the stimulation site, LCGU was increased in the ipsilateral cuneiform nucleus, parabrachial area, and locus coeruleus. Marked increases in LCGU were also noted in the thalamus and motor cortex contralateral to the paw used to depress the lever.

These data represent the first successful combination of the 2-DG technique (with appropriate quantification) and freely moving animals working for rewarding brain stimulation. The results indicate that rather than a widespread pattern of neuronal activation, this behavior involves discrete neuronal activation of DA projection fibers and selective terminal sites.

Stimulation of substantia nigra in animals pretreated with haloperidol increased glucose utilization in the ipsilateral globus pallidus, entopeduncular and subthalamic nuclei as compared to non-treated, stimulated animals. In non-stimulated animals, 6-OHDA lesions alone produced a slight increase in metabolic activity of the ipsilateral globus pallidus (20%). Nigral stimulation of 6-OHDA-treated animals did not increase glucose utilization in the entopeduncular or subthalamic nuclei. Not further increased by nigral stimulation, pallidal glucose metabolism remained the same as in 6-OHDA-lesioned animals without stimulation. Pretreatment with haloperidol in 6-OHDA-lesioned animals receiving nigral stimulation eliminated ipsilateral increases in glucose utilization in globus pallidus. Nigral dopamine neurons appear to be essential for metabolic activation of the entopeduncular and subthalamic nuclei in nigral stimulated animals. In contrast, the globus pallidus did not require such input. This finding suggests that elevated pallidal glucose metabolic activity is due either to the effects of dopamine denervation or stimulation of non-dopaminergic fibers. In 6-OHDA-lesioned animals, haloperidol may suppress metabolism in the globus pallidus by binding to cells rendered hypersensitive to this dopamine agonist.

Opiate receptors labeled with [^3H]naloxone in a mu ligand selectivity pattern showed striking laminar heterogeneity in rat cortex and were densest in limbic cortical areas, intermediate in the motor cortex, and fewest in the primary sensory areas. By contrast, opiate receptors labeled with [^3H] D-ala²-D-Leu⁵-enkephalin in a delta selectivity pattern are much more homogeneously distributed across both regions and laminae within regions.

Significance to Biomedical Research and the Program of the Institute

Since opiates are among the most potent psychotomimetic and euphorogenic compounds, it is of special significance that there exist endogenous compounds in brain which are apparently mimicked by opiates. There is substantial evidence that these compounds may be novel neurotransmitters or neuroregulators. If this is the case, then it is quite conceivable that such neurohumors may play an important role in affective states. Other peptides in brain appear to exert important effects on dopamine neurotransmission. Since dopaminergic dysfunctions have been related to mental disorders, it is possible that various neuropeptides may be involved in the etiology of such illnesses.

Publications

Quirion, R., Larsen, T.A., Calne, D., Chase, T., Rioux, F., St.-Pierre, S., Everitt, H.D., Pert, A., and Pert, C.B.: Analysis of [^3H]-neurotensin receptors by computerized densitometry: Visualization of central and peripheral neurotensin receptors. Ann. N.Y. Acad. Sci., 400: 415-417, 1982.

Bowen, W.D., Pert, C.B., and Pert, A.: Nigral 6-hydroxydopamine lesions equally decrease mu and delta opiate binding to striatal patches: Further evidence for a conformationally malleable Type 1 opiate receptor. Life Sci., 31: 1679-1683, 1982.

Lewis, M.E., Pert, A., Pert, C.B. and Herkenham, M.: Opiate receptor localization in rat cerebral cortex. J. Comp. Neurol., 216: 339-358, 1983.

Chiueh, C.C., Markey, S.P., Burns, R.S., Pert, A., and Kopin, I.J.: Changes in contents of dopamine, serotonin and their metabolites in the substantia nigra of rats following systemic and intranigral administration of N-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine. Eur. J. Pharmacol., 1983, in press.

Bragin, E., Moody, T.W., Pert, C.B., and Pert, A.: Effects of stress on brain and spinal cord levels of bombesin, substance P and endorphins. Pharmacol. Biochem. Behav., 1983, in press.

Hruska, R.E., Ludmer, L.M., Pert, A., and Bunney, W.E., Jr.: Effects of lithium on muscarinic cholinergic receptors. Life Sci., 1983, in press.

Hammer, D. and Pert, A.: Effects of opiates on the activity of dopaminergic and non-dopaminergic neurons in the substantia nigra. Peptides, 1983, in press.

Quirion, R., O'Donahue, T.L., Everitt, H., Pert, A., and Pert, C.B.: Pencyclidine receptors and possible existence of an endogenous ligand. Life Sci., 1983, in press.

O'Donahue, T.L., Pert, C.B., French, E.D., Pert, A., DiMaggio, D.A., Everitt, H., and Quirion, R.: Evidence for an endogenous central nervous system ligand for the pencyclidine receptor. Life Sci., 1983, in press.

Beinfeld, M.C., Lewis, M.E., Eiden, L.E., Nilaver, G., Pert, C.B., and Pert, A.: The distribution of cholecystokinin and vasoactive intestinal peptide in rhesus monkey brain as determined by radioimmunoassay. Peptides, 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01MH00081-09 BP

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Heritable Characteristics of Cation Transport in Primary Affective Disorders

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John I. Nurnberger, Jr., M.D., Ph.D., Medical Officer, BPB, NIMH

COOPERATING UNITS (if any)

Temple University, Philadelphia, Pennsylvania

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Psychogenetics

INSTITUTE AND LOCATION

NIMH, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.1

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Sodium potassium stimulated adenosine triphosphatase has been found to decrease in response to dextroamphetamine in normal volunteers. This is similar to a decrease found in the depressed state.

Specific binding of the ion pump blocker ouabain was measured in red cells and lymphocytes. We were unable to confirm reports that incubation in a low potassium medium should increase numbers of binding sites, and we are not pursuing this work.

The calcium pump co-factor protein calmodulin has been measured in cerebrospinal fluid of controls and euthymic bipolar patients. No difference has been found in the groups.

Project Description:

In collaboration with Dr. E. S. Gershon, Ms. S. Simmons-Alling and Ms. D. Lawrence of our section, we have continued our investigation of sodium potassium-stimulated adenosine triphosphatase in human erythrocytes. The activity of this enzyme was previously demonstrated to be related to mood state in manic-depressive illness (Nurnberger et al., 1982). We have examined pharmacologic agents to see if alterations similar to those in affective illness may be provoked. The monoaminergic agonist amphetamine (0.3 mgm/kg) produced a significant ($p = .02$) decrease in ATPase activity in 6 normal volunteers. The amount of decrease, however, was not significantly related to mood and behavioral response scores in the subjects.

We attempted replication of work by Naylor and colleagues indicating a difference between bipolar patients and controls in the control of ouabain-binding sites in peripheral lymphocytes. Their data showed a stimulation of production of ouabain-binding sites in peripheral lymphocytes by overnight incubation in a low potassium medium in normal controls but not in patients. In our studies on 4 volunteers we were unable to demonstrate the increased number of binding sites reported by Naylor. We have concluded that if there is such an effect it is not likely to be a potent determinant of bipolar disorder.

In collaboration with Dr. W. H. Berrettini of our section and Dr. W. Narrow of Temple University, we have studied the protein calmodulin, which has important regulatory functions in calcium transport and many other cellular functions, in cerebrospinal fluid from 25 bipolar patients and 25 normal controls. Calmodulin was measured by radioimmunoassay and found to be not different in the two groups. In 9 paired samples from patients on and off lithium no effect of lithium treatment was seen. Therefore, we cannot support a role for this regulatory protein in affective illness. However, this is the first report of calmodulin measurement in human spinal fluid, as far as we know.

Significance to Biomedical Research:

Cation transport mechanisms depend on cell membrane characteristics that may be largely genetically determined. We have demonstrated state dependent differences in cation transport mechanisms in affectively ill patients, but have not as yet identified trait differences.

Proposed Course of Project:

We have a continuing interest in markers of disordered cation transport in affective illness. Our own work has thus far not supported hypotheses regarding altered cation transport as a genetic vulnerability factor in affective illness. We are presently re-investigating the claim that altered lithium transport is present in a subgroup of bipolar patients following a recent report of new evidence.

Publication:

Nurnberger, J. I., Jr., Jimerson, D. C., Allen, J. R., Simmons, S., and Gershon, E. S.: Red cell ouabain sensitive Na^+/K^+ -ATPase: A state marker in affective disorder inversely related to plasma cortisol. Biol. Psychiatry 17: 981-992, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01MH00084-09 BP
PERIOD COVERED October 1, 1982 - September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Genetic-Biologic Studies of Psychiatric Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Elliot S. Gershon, M.D., Chief, Section on Psychogenetics, BPB, NIMH		
COOPERATING UNITS (if any) INSERM, University of Cagliari, University of Miami, Institute for Medical Research, Yale University, Washington University, LCS, NSB		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychogenetics		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">7.5</div>	PROFESSIONAL: <div style="text-align: center;">4.5</div>	OTHER: <div style="text-align: center;">3.0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Muscarinic cholinergic</u> and <u>beta-adrenergic neuronal characteristics in fibroblasts</u> continue to be investigated and applied to clinical issues. Cholinergic characteristics identified in fibroblasts include <u>choline acetyl transferase activity</u> and <u>high affinity choline uptake</u>. <u>Muscarinic receptors on fibroblasts</u> have <u>increased density after atropine incubation</u>, which returns to normal after atropine is removed. Cholinergic agonists produce inhibition of norepinephrine-stimulated adenylyl cyclase. <u>Beta-adrenergic receptors on fibroblasts</u> have significantly <u>reduced density in patients with affective illness and ill relatives</u>, which in combination with our previous finding of decreased muscarinic receptor density in these individuals, <u>supports the cholinergic-adrenergic balance hypothesis of affective disorders</u>. </p> <p> Analysis of <u>family and linkage data in pedigrees</u> shows <u>single locus control of platelet MAO activity</u>. A family study of <u>schizophrenia</u> has been undertaken. </p> <p> In the recombinant DNA laboratory, a <u>restriction fragment length polymorphism of pro-opiomelanocortin (POMC)</u> has been identified. </p>		

1. Family Study of Psychiatric Disorder

Methodologic study of diagnostic information from relatives revealed that with four or more informants only 2/3 of relatives with major affective diagnosis by direct examination are identified. Hypomania and schizoaffective disorder are least likely to be identified. This emphasizes the methodologic advantage of family study (direct examination of relatives) as compared with family history.

Data collection is now completed in the controlled study of childhood disorders in children of parents with affective disorder. Analysis of data is proceeding. Collaborators are Mrs. J. Hamovitz of this section and Dr. D. McKnew, Dr. L. Cytryn and Dr. W. Sceery of LCS.

Two new studies have been initiated:

A. A "high risk" study of offspring of bipolar patients (see Z01 MH 00086-07 BP)

B. Schizophrenia family study

Biologic-genetic data in schizophrenia has been meager. In collaboration with the Clinical Neurosciences Branch (Dr. D. Pickar and Ms. J. Schreiber), we have developed diagnostic and interview procedures for schizophrenia, schizophrenia spectrum disorders, and related disorders such as simple delusional disorders. We also plan to study inheritance of characteristics such as ventricular brain ratio in these families.

2. Genetic Analyses of Affective Disorders

A. Linkage to MNS

We previously reported that affective disorder was possibly linked to the MNS group locus. To further investigate this possibility, we pooled our informative families (approximately 30) with an additional 4 families from the Amish population collected by Dr. J. Egeland (University of Miami). We calculated lod scores under varying assumptions about the mode of inheritance and the definition of the ill phenotype. However, we were not able to confirm the hypothesis of linkage. Even though certain diagnostic classifications yielded positive linkage results within samples, there was no one definition consistent with linkage in the pooled sample.

B. Linkage to X-chromosome Markers

Our data have not supported the hypothesis of linkage of bipolar affective disorder to the colorblindness region of the X-chromosome. In collaboration with Dr. M. del Zompo at the University of Cagliari in Sardinia, we have analyzed X-linkage in two bipolar pedigrees, one informative for colorblindness and one informative for G6PD deficiency. These two pedigrees were consistent with the hypothesis of X-linkage with linkage being approximately

30 times more likely than no linkage. This small sample does not allow for a definitive conclusion but it indicates that more data should be collected, especially since the G6PD marker is highly polymorphic in this population.

C. Transmission of MAO Activity

We previously reported that MAO activity in our sample was not related to susceptibility to affective disorders. The transmission of MAO in families was consistent with several major locus hypotheses, whereas a strictly non-genetic hypothesis was rejected. These data have recently been reanalyzed in collaboration with Dr. J. Rice at Washington University, applying a mixed model of inheritance (which includes both a major locus and a polygenic component) to these data. He was able to reject dominant and purely additive inheritance. The acceptable models were: 1) codominant inheritance where the heterozygote distribution was displaced about 30% from the "low MAO" homozygous distribution; and 2) recessive inheritance with a polygenic background. In either case, the major locus accounted for about 30-40% of the total variance. However, the locus appears to cause "high" levels of MAO rather than "low" levels. That is, the majority of individuals have the genotype for low or normal MAO, so there is no major gene associated with the reduced MAO activity reported in several psychiatric disorders.

D. Theoretical Studies

We previously reported the results of simulation studies carried out in collaboration with Dr. K. Kidd at Yale University showing that a major locus not detectable by segregation analysis can sometimes be inferred if a closely linked marker locus is present. We have extended this study and found that the power of the linkage method is improved if the trait locus parameters (gene frequency and genotypic penetrances) are estimated jointly with the linkage parameters. Joint estimation of parameters is not generally carried out in real linkage studies. Our results suggest that it should be more commonly used.

3. Fibroblasts as a Neuronal Model

Fibroblasts: Using the same method as described in the previous report, fibroblasts were obtained from patients with manic-depressive illness, their ill and well relatives, normal volunteers, Alzheimer's, Huntington's and Gilles de la Tourette Syndrome patients. In addition, a pedigree of Amish manic-depressives was put into culture by Dr. J. Egeland.

The parameters studied in these cell lines were as follows: muscarinic binding and beta-binding; choline acetyl transferase activity; high affinity choline uptake; effects of exposure to drugs such as atropine, arecoline, lithium, cortisol on the binding of dihydroalprenolol and quinuclidinyl benzilate (QNB) on the fibroblasts. Dr. N. S. Nadi has been the principal investigator in this effort. Collaborators include Dr. J. I. Nurnberger, Jr. and Dr. W. H. Berrettini of this section, and Dr. A. Greene of the Institute of Medical Research.

Muscarinic binding: In the preceding report it was shown that fibroblasts contained a muscarinic "binding site" which fulfilled the criteria for a receptor as described by Cuatrecasas, including saturable and specific binding of QNB; displaced by atropine; linear with tissue concentration; pharmacologic properties of the site are similar to the brain. Norepinephrine-stimulated adenylate cyclase in the fibroblasts is inhibited by acetylcholine, arecoline, and oxotremorine. The culture characteristics of the receptor were studied and it was found that the B_{max} and K_d of the receptor were stable to generation 10.

The muscarinic binding was then compared between the normal volunteers, patients and their ill relatives. Twelve patients and 9 ill relatives had a B_{max} of 329.0 ± 63.9 and 294.8 ± 47.3 fmol/mg protein, respectively. This value was significantly higher than 12 normal volunteers (227.8 ± 47.3 fmol/mg protein). The level of significance was patient vs. control ($p < 0.001$) and ill relatives vs. control ($p < 0.02$). There was no significant difference between the ill relatives and the probands. No significant difference in K_d was observed. Further studies of patients, volunteers, and entire pedigrees are proceeding with 50 new specimens placed in culture this year.

Effects of in vitro exposure to atropine: $10^{-5}M$ atropine in the solution increased the B_{max} of 3H QNB binding but did not change the K_d . This effect is very similar to that observed in the brain. More interestingly, once atropine was removed from the medium and the cells were grown for one passage in its absence, the B_{max} returned to its pre-atropine levels. This suggests that the patient-control differences in B_{max} are not due to prior exposure to anticholinergic drugs.

Beta Binding: This binding was studied using 3H dihydroalprenolol as the ligand. As was previously known it fulfilled the requirements of a receptor. Our studies show that patients and ill relatives ($n = 21$, $B_{max} = 1.01 \pm 0.22$ pmol/mg protein) have lower B_{max} than normal volunteers ($n = 12$, 1.86 ± 0.19 pmol/mg protein; $p < 0.01$). The K_d is $10^{-12}M$ and is not significantly different between the two groups.

Choline Acetyltransferase Activity (CAT): Using 3H acetyl CoA as a substrate we were able to demonstrate, using the assay developed by Wilson et al., that the fibroblasts have a very small amount of CAT activity 153.0 ± 108.9 fmol/mg protein/15 minutes (5 cell lines, 2 separate measurements). A very small study of cells from patients with affective disorder showed no difference between them and controls ($n = 4$). In the studies involving cells from patients with Alzheimer's Disease, a downward trend was observed in 4 patients vs. 4 controls. More studies are underway.

High Affinity Choline Uptake: The existence of a high affinity choline uptake system was demonstrated in the fibroblasts and confirmed by us. Studies comparing this system in the affective patient population and the Alzheimer's patients' cells did not show any significant differences.

The opposite of the increase in receptor numbers was also studied by exposing the cells to cholinergic agonists. However, contrary to what is observed in some but not all regions of the brain, the cholinergic agonists arecoline and oxotremorine failed to induce a decrease in the B_{max} . The reasons for this difference are currently being investigated.

Effects of lithium: 10 M Li⁺ causes a decrease in the cholinergic receptor number on the fibroblasts, 24 hours after being added to the medium. The decrease in the fibroblasts from patients with affective disorder is much larger when compared to controls, 46 ± 10.5 vs. 9.45 ± 6.05 ($p < 0.001$), possibly indicating a differential regulation of the QNB receptors in patients vs. controls. Studies are under way of changes in rat CNS muscarinic receptors and CAT activity after Li feeding.

Other fibroblast receptors of interest in neurobiology: Careful studies have shown no saturable specific binding for the following receptors: dopamine, serotonin, GABA, glutamate, nicotinic cholinergic, somatostatin, angiotensin II, vasopressin, thyrotropin releasing hormone, adenocorticotropin, vasoactive intestinal peptide, neurotensin, and opiate.

4. Basic Neuroscience Studies (Dr. N. S. Nadi)

QNB Binding and Choline Acetyltransferase (CAT) in the Rat Brain Following Adrenalectomy

The imbalance in the pituitary adrenal axis of at least a subpopulation of patients with affective disorders is well known. We wanted to study the effects of adrenalectomy on the binding of ^3H QNB and activity of CAT in different areas of the rat brain. It was found that QNB decreased in the olfactory bulb $B_{max} = 500 \pm 124$ vs 1140 ± 130 fmol/mg protein control. The other brain areas did not show such changes. This change could be reversed by injecting the rats with dexamethasone. The K_d of the QNB binding was unaltered. No changes in the QNB B_{max} or K_d were seen following medullectomy. CAT activity showed no changes following adrenalectomy in the cortex, midbrain, cerebellum, hippocampus and amygdala. Dr. D. Jimerson of LCS has collaborated in this project.

Glutamate and Aspartate Receptors in the Cerebellum of the Rat: In these studies glutamate and aspartate receptors were identified in the cerebellum. It was shown, based on different displacements by quicalate, kainic acid, DL- α -amino adipic acid that these two receptors are different. This difference was further established by the destruction of climbing fibers, (which are presumably aspartate containing), upon which an increased B_{max} for aspartate was observed, but the B_{max} of glutamate binding was unaltered.

Recent studies in our laboratory have also shown that the cerebellum contains a peptide of approximately 500 MW which displaces glutamate binding. Studies are in progress towards the identification of this peptide.

5. Molecular Genetic Studies (Dr. P. V. Choudary)

Variation in human neuropeptide genes is of interest because of possible functional or pathological effects of the variation. A restriction fragment length polymorphism (RFLP) has been identified in the area of the gene coding for pro-opiomelanocortin (POMC). The site is identified by digestion of lymphocyte DNA with the restriction endonuclease Bam H₁, and hybridization to a cDNA probe for bovine POMC.

In a parallel clinical investigation, we are looking for possible association of specific polymorphisms with psychiatric illness or with altered quantities of POMC products in blood, as measured by RIA. These include the N-terminal peptide of POMC, ACTH, beta-endorphin, beta-LPH and α -MSH.

6. Other Studies (Dr. W. H. Berrettini)

Plasma gamma-amino-butyric acid (GABA) is consistently reduced in bipolar patients, and shows significant correlation between monozygotic twins. Dr. Post has collaborated in this work. A family study of plasma GABA is underway.

Significance to Biomedical Research and the Program of the Institute

Successful identification of a marker of genetic vulnerability to affective disorders would lead to identification of the responsible pathophysiological process, and would have clinical applications for prevention and choice of treatment. The findings on fibroblasts of increased density of muscarinic receptors, and decreased beta receptor density are very promising at this time. Further delineation of "neuronal" functions on the fibroblast contributes to the usefulness of the fibroblast as a clinical model of the neuron.

Studies of adolescents at risk for psychiatric illness, and family studies of schizophrenia, offer an opportunity to identify related clinical syndromes, and the chance to test clinical trait marker hypotheses.

The DNA laboratory is the first of its kind in clinical genetic investigation in psychiatry. The initial project has demonstrated a polymorphism in the gene for pro-opiomelanocortin, whose biological derivatives include ACTH or beta-endorphin. Clinical and physiological studies of this gene are proceeding.

Proposed Course of Project

We plan to continue to investigate the biology and genetics of characteristics that may be implicated in the genetics of affective disorders, as described above. Further study of the fibroblast as a clinical neuronal model will proceed. The molecular genetics approach to interindividual differences in neuropeptides and other substance will be pursued. Establishing a library of DNA and living cells from entire

pedigrees is a major priority. Study of relatives at risk for affective disorders and schizophrenia will proceed. Mathematical methodology for clinical investigation will continue to be studied.

Publications:

Goldin, L.R., Gershon, E.S., Targum, S.D., Sparkes, R.S., and McGinniss, M.: Segregation and linkage analyses in families of patients with Bipolar, Unipolar, and Schizoaffective mood disorders. Am. J. Hum. Genet. 35: 274-287, 1983.

Gershon, E.S., Hamovit, J., Guroff, J.J., Dibble, E., Leckman, J.F., Sceery, W., Targum, S.D., Nurnberger, J.I., Goldin, L.R., and Bunney, W.E., Jr.: A family study of Schizoaffective, Bipolar I, Bipolar II, Unipolar, and normal control probands. Arch. Gen. Psychiatry 39: 1157-1167, 1982.

Goldin, L.R., Clerget-Darpoux, F. and Gershon, E.S.: Relationship of HLA to major affective disorder not supported. Psychiatry Res. 7: 29-45, 1982.

Clerget-Darpoux, F., Goldin, L.R., and Gershon, E.S.: A new methodology for analysis of HLA-associated diseases? Letter to the editor. Am. J. Hum. Genet. 35: 127-130, 1983.

Choudary, P.V. and Ramananda Rao, G. The free amino acid pool in Candida albicans contains higher levels of glycine and methionine than in non-pathogenic Candida species. Microbios Letters. in press.

Choudary, P.V.: Susceptibility of host brain is antecedent to mortality in experimental candidiasis. Microbios Letters. in press.

Apter, A., Borengasser, M.A., Hamovit, J., Bartho, J., Cytryn, L., and McKnew, D.H.: A four-year follow-up of depressed children. J. Prev. Psychiatry, in press.

Berrettini, W.H., Nadi, N.S., and Gershon, E.S.: Absence of specific binding of several putative neuro-transmitters to human fibroblasts. J. Recept. Res., in press.

Berrettini, W.H., and Post, R.M.: GABA in Affective Illness. In Post, R.M. and Ballenger J.C. (Eds.): Neurobiology of Mood Disorders, Baltimore, Maryland, Williams and Wilkins, in press.

Clerget-Darpoux, F.: Bias of the estimated recombination fraction and lod score due to an association between a disease gene and a marker gene. Ann. Hum. Genet. 46: 363-372, 1982.

Gershon, E.S.: Genetics of Major Psychoses. In Kety, S.S., Rowland, L.P., Sidman, R.L. and Matthysse, S.W. (Eds.): Genetics of Neurological and Psychiatric Disorders, New York, Raven Press, 1983, pp. 121-144.

Gershon, E.S.: Genetic Studies of Affective Disorders and Schizophrenia. In Bonne-Tamir, B., Cohen, T., and Goodman, R.M. (Eds.), Human Genetics, Part A: The Unfolding Genome, New York, Alan R. Liss, Inc., 1982, pp. 417-432.

Gershon, E.S.: Should science be stopped: The case of recombinant DNA research. The Public Interest 71: 3-16, 1983.

Gershon, E.S.: What is the familial morbid risk of major depression? Letter to the Editor, Arch. Gen. Psychiatry, in press.

Gershon, E.S., Goldin, L.R., Weissman, M.M., and Nurnberger, J.I., Jr.: Family and genetic studies of affective disorders in the Eastern United States: A provisional summary. In Jansson, B., Perris, C. and Struwe, S. (Eds.), Proceedings of the IIIrd World Congress on Biological Psychiatry, Amsterdam, Elsevier Press, in press.

Gershon, E.S. and Guroff, J.J.: Information from relatives: Diagnosis of affective disorders. Arch. Gen. Psychiatry, in press.

Gershon, E.S., Hamovit, J.R., Schreiber, J.L., Dibble, E.D., Kaye, W., Nurnberger, J.I., Jr., Andersen, A., and Ebert, M.: Anorexia nervosa and major affective disorders associated in families: A preliminary report. In Guze, S.B., Earls, F.J., and Barrett, J.E. (Eds.): Childhood Psychopathology and Development, New York, Raven Press, 1983, pp. 279-286.

Goldin, L.R., Gershon, E.S. and Belmaker, H.: HLA and affective disorders (Editorial) (Hebrew). Harefuah, in press.

Nadi, N.S., and Getchell, T.V.: Antidromic activation of receptor neurons in the salamander. Brain Res., in press.

Nadi, N.S., Getchell, M.L., and Getchell, T.V.: Properties of odor recognition and impulse initiation membranes of olfactory receptor neurons. Brain Res., in press.

Nadi, N.S., and Margolis, F.L.: Some observations on the transsynaptic regulation of the levels of neurotransmitters in the CNS. Science, in press.

Nadi, N.S., and Shephard, G.: The olfactory system: Review of its biochemistry, anatomy and physiology. Ann. Rev. Neuroscience, in press.

Nurnberger, J.I., Jr., Jimerson, D.C., and Bunney, W.E., Jr.: A risk factor strategy for investigating affective illness. Biol. Psychiatry, in press.

Schulz, P., Schulz, C., Dibble, E., Targum, S., van Kammen, D., and Gershon, E.S.: Patient and family attitudes about schizophrenia: implications for genetic counseling. Schizophrenia Bull. 8: 504-514, 1982.

Nurnberger, J.I., Jr.: Manic-depressive illness. In Myrianthopoulos, N.C. (Ed.): Handbook of Clinical Neurology, Amsterdam, Elsevier, pp. 208-210, 1982.

Weissman, M.M., Gershon, E.S., Kidd, K.K., Prusoff, B.A., Leckman, J.F., Dibble, E., Hamovit, J., Thompson, W.D., Pauls, D., and Guroff, J.J.: Psychiatric disorders in the relatives of probands with affective disorders: The Yale-NIMH collaborative family study. Arch. Gen. Psychiatry, in press.

Gershon, E.S., Nurnberger, J.I., Jr., Nadi, N.S., Berrettini, W.H., and Goldin, L.R.: Current status of genetic research in affective disorders. In Angst, J. (Ed.): The Origins of Depression: Current Concepts and Approaches. Dahlem Konferenzen 1982, Berlin, Springer-Verlag, in press.

Gershon, E.S.: Genetic perspectives. In Goodwin, F.K. and Jamison, K.R. (Eds.): Manic-Depressive Illness, Oxford, England, Oxford University Press, in press.

Gershon, E.S., Nurnberger, J.I., Jr., and Berrettini, W.H.: Genetic markers in affective disorders. Proceedings of the Congress of the Collegium Internationale Neuropharmacologicum (CINP), Jerusalem, Israel, June 1982, in press.

Nurnberger, J.I., Jr. and Gershon, E.S.: Genetics of affective disorders. In Post, R., and Ballenger, J. (Eds.): Neurobiology of Mood Disorders, Baltimore, The Williams & Wilkins Co., in press.

Clerget-Darpoux, F.: Analysis for HLA association with diseases: terminology, methodology, and interpretation. In Schwartz, L.M. (Ed.): Compendium of Immunology, Vol. 4, New York, Von Nostrand Reinhold Company, in press.

Nurnberger, J.I., Jr. and Gershon, E.S. Genetics of affective disorders. In Friedman, E. (Ed.): Depression and Antidepressants: Implications for Cause and Treatment. New York, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01MH00085-07 BP
PERIOD COVERED October 1, 1982 - September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pharmacogenetics of Psychoactive Drugs		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) John I. Nurnberger, Jr., M.D., Ph.D., Medical Officer, BPB, NIMH		
COOPERATING UNITS (if any) University of Oregon		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychogenetics		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">0.9</div>	PROFESSIONAL: <div style="text-align: center;">0.1</div>	OTHER: <div style="text-align: center;">0.8</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) <p>The behavioral excitation response to amphetamine has been found to be blocked by haloperidol but not by propranolol or thymoxamine; this suggests specific dopaminergic mediation of this response in normal man. The norepinephrine and pressor responses, on the other hand, are blocked by propranolol, suggesting mediation through beta-adrenergic receptors. None of the blocking agents studied significantly blocked the hormonal responses to amphetamine. Haloperidol caused a significant potentiation of the prolactin response.</p> <p>A pilot study of the effects of intravenous gamma amino butyric acid was performed in 6 patients and 5 normal controls. A number of subjects experienced dysphoric effects. No significant effects on hormone levels were seen.</p> <p>Eleven bipolar patients and 14 controls participated in a study of sensitivity of melatonin secretion to suppression by light. Euthymic patients were found to be more sensitive than controls; this may be a trait marker for vulnerability to depression.</p>		

Project Description

1. Pharmacologic dissection of amphetamine responses.

Together with Dr. E. S. Gershon and Ms. S. Simmons-Alling of our section, we have continued our studies of genetic aspects of behavioral and biochemical responses to drugs that perturb various neurotransmitter systems. We had determined through twin studies that the behavioral excitation response to amphetamine was concordant in monozygotic twins, reproducible in an individual over time, and uncorrelated with plasma amphetamine level. Using the same system for blind rating of videotapes that we have described previously, we analyzed data from 40 dextroamphetamine infusions done in 10 normal volunteers with and without neurotransmitter receptor blocking agents. Ratings were performed by Ms. S. Jimerson, Dr. E. Hostetler and Dr. L. Kessler of our section, and Ms. J. Schreiber of the Neuroscience Branch. Excitation was found to be blocked by haloperidol but not by propranolol or thymoxamine, corroborating our previous suggestion that this response might be related to inherited variation in sensitivity of dopamine receptors.

Systolic blood pressure response to amphetamine was attenuated by propranolol as was the rise in norepinephrine (assayed by Dr. D. Goldstein, NHLBI). This suggests a role for beta-adrenergic receptors, either post-synaptic or pre-synaptic in amphetamine-induced hypertension.

Cortisol response (measured by Dr. N. S. Nadi of our section) was nonsignificantly attenuated by the alpha-adrenergic blocker thymoxamine. Growth hormone response (measured by Dr. C. Tamminga, NINCDS) was not significantly affected by any of the three blocking agents. Prolactin response was potentiated by haloperidol, suggesting a synergism between amphetamine and haloperidol in prolactin release. Neurochemical mechanisms controlling endocrine responses to amphetamine cannot yet be characterized, but are apparently not related to the behavioral excitation response. A future study employing the serotonergic blocker metergoline is planned and other systems, such as the endogenous opiate system, may be investigated.

2. Responses to intravenous GABA

We have reported that gamma amino butyric acid (GABA) is lower in plasma for euthymic bipolar patients than in controls, and that lithium treatment appears to raise plasma GABA back to normal levels. In collaboration with Dr. W. H. Berrettini, Ms. S. Simmons-Alling and Dr. N. S. Nadi of our section, we investigated the effects of intravenous GABA on euthymic bipolar patients off medications and normal controls. Twenty infusions of various doses of GABA (0.1 to 1.0 mg over 5 minutes) were administered to 6 patients and 5 controls. Dose-related increases in pulse ($r = 0.45$, $p < .05$) and systolic blood pressure ($r = 0.47$, $p < .05$) were seen along with dysphoria in most subjects. Initial analysis of hormonal responses shows that cortisol levels fell, but not significantly more than after

placebo, and growth hormone and prolactin levels did not change. We do not have any indication that intravenous GABA responses will provide a useful genetic vulnerability marker for affective disorder.

3. Light suppression of melatonin

In collaboration with the Clinical Psychobiology Branch, we are studying another "pharmacologic" response in which light is used as a drug. In this paradigm, developed by Dr. Alfred Lewy, blood samples are drawn from subjects at night before and after stimulation by a light of moderate (500 lux) intensity. The light will cause reduction in plasma melatonin. We had previously reported on increased sensitivity to light in 5 euthymic bipolar subjects as compared with 6 normal controls; there was a question as to whether season of testing skewed that data. We have now expanded that series, controlling for any seasonal effect. In collaboration with Dr. A. Lewy, University of Oregon and Dr. T. Wehr, CPB, NIMH, eleven euthymic bipolar patients off medications have been tested and compared with 24 controls. Patients are more sensitive to the effects of light ($p < .005$ by Mann Whitney U test). Work in rats suggests that the response to light may be mediated by acetylcholine. We currently plan to try pharmacologic manipulation of this response with cholinergic agonists and antagonists.

Significance

Using these methods we have identified several responses that may be markers for the genetic vulnerability to affective disorder (the cholinergic REM induction test and the light-melatonin response).

Our pharmacogenetic studies of amphetamine have provided basic data about drug-induced behavioral variation in man and its neurochemical substrate. The excitation response may be useful as a tool for assessing individual dopaminergic sensitivity.

Proposed Course of Project

The cholinergic REM induction studies are being restarted; we intend to apply this test and the test of light-melatonin sensitivity to offspring of bipolar patients being recruited for the high risk study as described in Z01 MH 00086-07 BP.

We plan a dose response study of amphetamine followed by an assessment of the effects of very low doses (1 - 3 mg) on REM sleep. A study of the effects of the serotonin antagonist metergoline on the hormonal responses to amphetamine is also planned.

Two new pharmacologic challenge studies related to hypothesized neuroendocrine abnormalities in primary affective disorder are planned. We propose to study the effects of naloxone on ACTH secretion in euthymic

bipolar patients and normal controls. We also plan to study TSH response to TRH in euthymic bipolars with no history of lithium treatments or in high risk subjects.

Publications

Sitaram, N., Kaye, W. H., Nurnberger, J. I., Jr., Ebert, M. H., Gershon, E. S., and Gillin, J. C.: Cholinergic REM sleep induction - A trait marker of affective illness? In Hanin, I. and Usdin, E. (Eds.): Biological Markers in Psychiatry and Neurology, Pergamon Press, N.Y., pp. 397-404, 1982.

Sitaram, N., Nurnberger, J. I., Jr., Gershon, E. S., and Gillin, J. C.: Cholinergic regulation of mood and REM sleep: Potential model and marker of vulnerability to affective disorder. Am. J. Psychiatry 139: 571-576, 1982.

Nurnberger, J. I., Jr., Gershon, E. S., Simmons, S., Ebert, M., Kessler, L. R., Dibble, E. D., Jimerson, S. S., Brown, G. L., Gold, P., Jimerson, D. C., Guroff, J. J., and Storch, F. I.: Behavioral, biochemical and neuroendocrine response to amphetamine in normal twins and "well state" bipolar patients. Psychoneuroendocrinology 7: 163-176, 1982.

Goldstein, D. S., Nurnberger, J. I., Jr., Simmons, S., Gershon, E. S., Polinsky, R., and Keiser, H. R.: Effects of injected sympathomimetic amines on plasma catecholamines and circulatory variables in man. Life Sci. 32: 1057-1063, 1983.

Nurnberger, J.I., Jr., Sitaram, N., Gershon, E.S. and Gillin, J.C.: A twin study of cholinergic REM induction. Biol. Psychiatry, in press.

Nurnberger, J.I., Jr., Jimerson, D.C., Simmons-Alling, S., Tamminga, C., Nadi, N.S., Lawrence, D., and Gershon, E.S.: Behavioral, physiological, and neuroendocrine responses to arecoline in normal twins and "well state" bipolar patients. Psychiatry Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01MH00086-07 BP
PERIOD COVERED October 1, 1982 - September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Outpatient Clinic for Genetic and Pharmacological Studies of Affective Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Elliot S. Gershon, M.D., Chief, Section on Psychogenetics, BPB, NIMH		
COOPERATING UNITS (if any) Clinical Research Institute of Montreal, Eunice Kennedy Shriver Center, New England Medical Center, Eli Lilly Pharmaceuticals, Jefferson University, Catholic University, University of Virginia, NIA		
LAB/BRANCH Biological Psychiatry Branch		
SECTION Section on Psychogenetics		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">2.9</div>	PROFESSIONAL: <div style="text-align: center;">2.2</div>	OTHER: <div style="text-align: center;">0.7</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>We have examined various parameters related to <u>hypothalamic-pituitary-adrenal axis</u> functioning in <u>euthymic bipolar patients</u> and controls. No differences were found in mean values of <u>urinary free cortisol</u>, <u>plasma cortisol</u> or <u>CSF cortisol</u>. Levels of <u>plasma</u> and <u>CSF proopiomelanocortin</u> were also <u>not different</u> in the two groups. It appears that <u>cortisol dysfunction</u> may be a <u>state</u> but not a <u>trait marker</u> for <u>affective illness</u>.</p> <p><u>Lumbar punctures</u> have been performed in 23 bipolar patients and a similar number of controls. Ten patients were studied both on and off lithium. Multiple neuroendocrine/biochemical parameters have been measured, but to date, <u>no difference between patients and controls</u> has been found. <u>Lithium effects on HVA and VIP</u> have been noted.</p> <p>The <u>enzyme hexosaminadase A</u> was measured in 30 bipolar patients from our clinic. One patient was found to have significant elevation of the enzyme.</p> <p>Five patients have participated in the <u>antidepressant study of pirlbuterol</u>. No sustained antidepressant effect was seen.</p> <p>Ten patients have participated in a study of <u>cognitive functions</u> in affective illness on and off lithium. We expect to test the effect of <u>DGAVP (des-glycyl-arginine vasopressin)</u> in some of these patients.</p> <p>A population of <u>offspring of bipolar patients</u> is currently being assembled for a <u>high risk study</u> of affective disorder. Biological measurements will be done and follow-up initiated over a period of years.</p>		

Project Description:

We maintain an ongoing treatment clinic for 100 manic-depressive outpatients for the purpose of: 1) identifying potential markers of genetic vulnerability to affective disorder; and 2) studying the course and treatment of affective illness, especially bipolar disorder. The clinic is administrated by Dr. J. I. Nurnberger, Jr.; patients are treated by Ms. S. Simmons-Alling, Dr. E. S. Gershon, Dr. W. H. Berrettini and Mrs. J. Hamovit.

For purposes of comparison, we have 52 normal volunteers and 23 pairs of monozygotic twins.

1. Markers of genetic vulnerability to affective illness

In contrast to most investigators in the field, we study primarily "well state" patients to determine those abnormalities that are most likely abiding characteristics of the illness.

A number of studies have identified abnormalities in the hypothalamic-pituitary-adrenal axis during the depressed state. To assess whether some aspect of this disturbance might be a "trait" marker we have studied the following parameters in collaboration with Dr. J. I. Nurnberger, Jr., Dr. W. H. Berrettini, Dr. N. S. Nadi, Ms. S. Simmons-Alling and Dr. P. V. Choudary, all whom are members of the Section on Psychogenetics, and Dr. M. Chretien of the Clinical Research Institute of Montreal.

- 1) urinary free cortisol in 20 patients and 15 volunteers;
- 2) plasma cortisol in 20 patients and 15 volunteers;
- 3) CSF cortisol in 15 patients and 17 volunteers;
- 4) the ACTH and beta-endorphin precursor proopiomelanocortin in plasma and CSF from 20 patients and 20 volunteers;
- 5) the proopiomelanocortin breakdown product α -MSH in CSF from 20 patients and 20 volunteers.

Further studies on associated peptides will include assessment of beta-endorphin, ACTH and beta-lipotropin. To date we have not found differences between patient and control groups.

We have performed a series of outpatient lumbar punctures on 25 euthymic bipolar patients during lithium treatment (12 of whom returned for unmedicated studies) and on 25 normal volunteers as part of an effort to search for trait factors in bipolar illness. This is the first outpatient lumbar puncture protocol at NIMH. It demonstrates the feasibility of conducting such studies in a less expensive, less time-consuming outpatient mode. Simultaneous blood samples were obtained in all cases. We studied the following substances (collaborators in parentheses): dopamine; dopamine SO_4 ; 5-HIAA, HVA, DOPAC, MHPG, NE (Dr. Linnoila, NIA); somatostatin (Dr. Reichlin, New England Medical Center); vasopressin (Dr. Zerbe, Eli Lilly Pharmaceuticals); vasoactive intestinal peptide [VIP]; neurotensin; cortisol; α -melanocyte stimulating hormone; beta-lipotropin [beta-LPH]; GABA (Dr. Hare, Jefferson University); and calmodulin.

Additionally, we have plans to measure acetylcholine ACTH and beta-endorphin (Drs. Chrousos, NICHD and Gold, BPB, NIMH) and dynorphin (Dr. Zamir, LCS, NIMH). To date none of the substances measured differentiated unmedicated euthymic bipolar patients from controls (see table). However, there were a number of interesting observations: 1) lithium decreases CSF and plasma VIP and increases CSF HVA. 2) high correlations were observed between CSF somatostatin and VIP; GABA and vasopressin; 5-HIAA and HVA, in all three groups studied. 3) no relationship was observed between CSF and plasma levels for GABA, α -MSH or VIP.

Comparison of our results with those of Drs. Rubinow (BPB, NIMH), Ballenger (University of Virginia), and Post (BPB, NIMH) indicate similar values can be obtained for some substances (5-HIAA) but not others (somatostatin). Issues concerning hours of bedrest, physical activity, emotional effects of hospitalization and diet may be involved in different normal ranges for some substances. Assay differences are probably not involved as the assays were performed by the same laboratory personnel on the same day (in the case of somatostatin).

CSF Biochemistry in Euthymic Bipolars and Controls

	Controls n = 20		Unmedicated Bipolars n = 10		Lithium-treated Bipolars n = 20	
Monoamines (pmol/ml)						
NE	0.32	+	0.20	0.34	+	0.21
MHPG	45.3	+	7.9	44.2	+	7.0
Dopamine	3.4	+	1.9	4.4	+	1.4
Dopamine-SO ₄	4.1	+	1.7	4.1	+	1.4
HVA*	180.0	+	54.0	178.0	+	72.0
DOPAC*	2.6	+	0.9	2.3	+	0.9
5-HIAA*	75.0	+	20.0	92.3	+	41.4
Peptides/Hormones (pg/ml)						
POMC	427.0	+	19.4	457.0	+	228.0
β-LPH	77.0	+	25.0	87.0	+	36.0
α-MSH	8.7	+	4.2	8.8	+	3.2
Cortisol	5800.0	+	2000.0	6700.0	+	1600.0
VIP	17.2	+	8.4	20.2	+	7.6
SRIF*	33.1	+	14.0	43.8	+	18.0
AVP	4.7	+	1.0	4.7	+	2.1
Calmodulin	29.9	+	15.0	26.1	+	17.4
Neurotensin	58.0	+	13.0	56.0	+	13.0
GABA** (pmol/ml)						
	127.0	+	50.0	129.0	+	36.0
	134.0	+	32.0			

Values are mean \pm S.D.

No significant ($p < .01$) group differences were found.

* corrected for height and weight

**corrected for age

A study of the enzyme hexosaminadase A has been performed. This enzyme is present at much reduced levels in carriers of Tay-Sachs Disease (heterozygotes) and it has been observed that some such carriers develop symptoms similar to those in bipolar illness. To assess how common this condition was in a defined bipolar population, plasma samples from 30 patients were obtained and sent to the Tay-Sachs Laboratory of the Eunice Kennedy Shriver Center, to be assayed by Dr. Ed Kolodny. Only one patient was noted to be heterozygotic for this trait.

3. Studies of the course and treatment of affective illness.

We have had a continuing interest in the treatment of depression in the course of bipolar illness. The clinical dilemma is that use of the standard antidepressants is associated with hypomania or mania in many bipolar patients and with increased frequency of depression in others. For this reason we are testing putative antidepressants with non-standard neurochemical profiles.

Our general protocol is an 8 week double-blind trial of active antidepressant versus placebo in patients maintained on lithium (the rationale being that lithium has antidepressant properties of its own). Hypomanic symptoms are assessed as well as depressive symptoms.

We have now tested 11 patients on the anticholinergic agent artane (with Dr. D. Jimerson, LCS, NIMH), 5 patients on the beta-2-adrenergic agonist pirbuterol (with Dr. L. Kessler, BPB, NIMH), 5 patients on the bicyclic serotonin reuptake inhibitor zimelidine (with Dr. W. Potter, CPB, NIMH), and 3 patients on placebo. Our preliminary conclusions at this point are that none of these agents is consistently helpful in our population. Certain individuals, however, have done well and have been maintained on artane and zimelidine.

A common complaint of lithium-treated patients is difficulty with memory and concentration. We are endeavoring to assess this complaint in collaboration with the Dr. B. Strupp and Dr. P. Gold, both from BPB, and Dr. H. Weingartner, LPP and Dr. B. Parry, CPB. Ten clinic patients have undergone cognitive testing on lithium and of these, one has so far completed similar tests during a medication free period. In general patients have not manifested a notable cognitive deficit. The vasopressin analogue DDAVP has previously been shown to enhance memory in normal volunteers by Dr. Weingartner and colleagues. are planning to study the related compound DGAVP in patients.

4. High risk study of affective disorder.

We are undertaking a prospective follow-up study of young persons (age 15-25) at high risk for affective disorder. High risk persons will be defined by the presence of bipolar illness in a parent. A control group of young persons without an ill parent will also be studied. A third group of persons with affective illness in both parents will also be sought. Information from our family study of affective illness indicates that 1 out of 4 persons with a bipolar parent will themselves

become ill; approximately 1 out of 2 persons with two ill parents will develop affective disorder. In our control group of families the incidence of severe affective disorder was about 7%. Almost half of those persons who do develop affective illness will have their onset between the ages of 15 and 25.

We propose to ascertain 50-100 young people in the high risk and control groups over the next several years. We will apply biologic studies that may be predictive, including for instance fibroblast muscarinic, and beta-adrenergic receptor binding (see below), cholinergic REM sleep induction, and light suppression of melatonin secretion (Z01 MH 00085-07). Follow-up studies, to be conducted over a period of about 10 years, will concentrate on clinical and psychosocial assessments (in collaboration with Dr. David Pellegrini of Catholic University).

Immediate and long-term outcome measures will be available. The initial biologic testing should, we hypothesize, differentiate high risk and low risk groups. Genetically vulnerable individuals should show the same abnormalities that we have demonstrated in persons in remission from bipolar illness. In addition, we expect to observe the onset of illness in some individuals over the course of the follow-up. The follow-up design should provide corroboration of the initial biologic results and enable us to assess the role of psychosocial stresses and protective factors in the onset of affective disorder.

Significance to Biomedical Research and the Program of the Institute

The marker studies are aimed at uncovering indicators of genetic vulnerability as discussed in Z01 MH 00085-07 BP. These might then lead us to a better understanding of the etiology of these conditions and enable early identification and monitoring of vulnerable persons.

The antidepressant trials are directed toward the solution of a difficult clinical problem as well as an improved neurochemical understanding of the switch process in bipolar illness.

The high risk study may provide confirmatory evidence for biochemical hypotheses regarding the etiology of manic-depressive illness. If a genetic vulnerability factor or factors can be demonstrated, new treatment strategies may be designed. In addition, clinical tools for the early identification of persons vulnerable to affective illness may be forthcoming. Presently available pharmacologic or psychosocial interventions might then be utilized to prevent the social deterioration that may result from untreated affective disorder.

Proposed Course of Study

Several measurements remain to be made on CSF from patients and controls, including ACTH, beta-endorphin, acetylcholine, and corticotropin releasing factor (CRF).

We are planning a new study of the experimental antidepressant bupropion. Recruitment for the high risk study is beginning and methods of data collection are being designed.

Publications:

Berrettini, W.H., Nurnberger, J.I., Jr., Post, R.M. and Gershon, E.S.: Platelet ^3H -imipramine binding in euthymic bipolar patients. Psychiatry Res. 7: 215-219, 1982.

Berrettini, W.H., Nurnberger, J.I., Jr., Worthington, E.K., Simmons-Alling, S., and Gershon, E.S.: Platelet vasopressin receptors in bipolar affective illness. Psychiatry Res. 7: 83-86, 1982.

Berrettini, W.H., Umberkoman-Wiita, B., Nurnberger, J.I., Jr., Vogel, W.H., Gershon, E.S., and Post, R.M.: Platelet GABA-transaminase in affective illness. Psychiatry Res. 7: 255-260, 1982.

Berrettini, W.H., Nurnberger, J.I., Jr., Hare, T., Gershon, E.S., and Post, R.M.: Plasma and CSF GABA in affective illness. Brit. J. Psychiatry 141: 483-488, 1982.

Nurnberger, J.I., Jr.: Manic Depressive Illness (Primary Affective Disorder). In Myrianthopoulous, N.C. (Ed.): Handbook of Clinical Neurology, Neurogenetic Directory, 1982, Part II, Volume 43, pp. 208-210.

Selinger, D., Simmons, S., Hailer, A.W., Nurnberger, J.I., Jr., and Gershon, E.S.: An effective method for measuring salivary lithium in patients on anticholinergic drugs. Biol. Psychiatry 17: 1145-1155, 1982.

Berrettini, W.H., Nurnberger, J.I., Jr., Hare, T.A., Simmons-Alling, S., Gershon, E.S., and Post, R.M.: Reduced plasma and CSF GABA in affective illness: Effect of lithium carbonate. Biol. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00132-09 BP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological and Psychopharmacological Evaluation of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation) Daniel P. van Kammen, M.D., Ph.D., Unit Chief,
Section on Neuropsychopharmacology, BP, NIMH

COOPERATING UNITS (if any) Arthritis and Rheum. Br, NIAMDD; Nuclear Medicine Dept, CC;
Hypertension-Endocrine Br, NHLBI; Lab Clin Psychopharmacology, NIMH; Lab Clin
Science, NIMH; Lab Psychology and Psychopathology, NIMH, Lab Clin Psychobiology,
NIMH

LAB/BRANCH

Biological Psychiatry Branch

SECTION

Section on Neuropsychopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, MD 20205

TOTAL MANYEARS:

14

PROFESSIONAL:

11

OTHER:

3

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

THIS PROJECT HAS BEEN TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 00326-10 CN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical neuropharmacology and psychobiology of depression and mania		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Dennis L. Murphy Chief, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) CN, BP, CPB, LCS, NIMH; Uniformed Services; VA Med. Cen., Bronx, NY; Naval Med. Cen.; UCLA; NIB, IRP, NINCDS		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.6	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> New evidence from several sources suggests that therapeutic responses to <u>monoamine oxidase-inhibiting antidepressants</u> occur in conjunction with reduced <u>sympathetic outflow</u> from the central nervous system. These <u>noradrenergic neurotransmitter system</u> changes develop only after longer-term drug administration. Depressed patients as a group have some corresponding evidence of enhanced sympathetic activity, including elevated plasma <u>norepinephrine</u> levels and, in a smaller subgroup of patients, an insensitivity to <u>post-synaptic adrenergic agonists</u>. At the same time, differences in <u>cortisol</u> responses to <u>clonidine</u> in depressed patients compared to normals indicated that <u>normalization of elevated plasma cortisol concentrations</u> present in some depressed patients can be achieved by clonidine, an α_2-noradrenergic agonist which does not reduce cortisol in normals--raising the question of a link between cortisol hypersecretion and noradrenergic dysfunction in depression. Other studies completed this year have examined additional elements in noradrenergic and <u>serotonergic receptor function</u> and neuroendocrine responsivity in depression, and have begun to evaluate possible common biological factors and treatment responses which might be present in patients with other diagnoses besides primary affective disorders. </p>		

Other collaborative professional personnel engaged on the project:

B. Roy	Staff Psychiatrist	CN NIMH
R. Lake	Staff Psychiatrist	Uniformed Services
L. J. Siever	Staff Psychiatrist	VA Med. Cen., Bronx
T. W. Uhde	Staff Psychiatrist	BP NIMH
J. A. Hamilton	Staff Psychiatrist	CN NIMH
B. Parry	Staff Psychiatrist	CPB NIMH
B. Gerner	Assoc. Professor	UCLA
S. P. Markey	Section Chief	LCS NIMH
L. Poth	Staff Psychiatrist	Naval Med. Cen.
R. Lowenstein	Staff Psychiatrist	BP NIMH
H. McFarland	Asst. Chief	NIB,IRP, NINCDS
J. Greenstein	Sen. Staff Fellow	NIB,IRP NINCDS
J. Rose	Sen. Staff Fellow	NIB,IRP NINCDS

Project Description:

Objectives: Individuals with depression, mania, and related disorders with affective components, including individuals with schizoaffective and characterologic disorders, are studied in attempts to understand the psychological and biological mechanisms involved in therapeutic drug effects in these disorders. As individual differences in psychoactive drug responsiveness appear to depend upon many psychological and biological factors, a variety of study approaches are utilized.

Methods Employed:

1. Behavioral and psychological assessment: Pretreatment evaluation of patients requires information from interviews of the patient and family, from psychometric approaches, from direct behavioral observation using various quantitative scales and from patients' self-ratings. The elucidation of individual and patient subgroup differences in drug response depends upon this information obtained by the clinical staff, including psychiatrists, psychologists, social workers and nursing personnel. Subsequent evaluation of drug response depends upon objective behavioral assessment as well as self-rated psychological change as obtained from a number of quantitative scales, several of which have been developed in this Branch.

2. Biological assessment: Pharmacologic challenge tests of central neurotransmitter systems are used to evaluate the functional state of these systems in patients compared to controls, and in patient groups studied before and during antidepressant drug treatment. Neuroendocrine, cardiovascular and behavioral changes are used as endpoints in these studies. Plasma, platelets, urine, and cerebrospinal fluid are collected for the measurement of biogenic amines and their metabolites, enzymes, other chemical variables, and drug levels. Electrophysiologic measurement of sleep and psychophysiological variables are accomplished in collaborative studies with investigators in other Branches.

Major Findings:

Our last year's work in the area of the affective disorders represents several different experimental approaches. As noted below and in the other project reports from the Branch, our group has been steadily broadening its clinical population focus, and correspondingly admitting fewer patients with major depressive disorders. At the same time, we have been very interested in affective elements present at the clinical phenomenological level as well as in studying biological and psychological factors which have been generally linked to depression but which seem to occur in at least some subgroups of patients with other diagnoses. The most clearly delineated comparisons accomplished within the Branch at present are those involving obsessive-compulsive patients (see project report Z01 MH 00336-04), some of whom manifest secondary depressive symptoms and some of whom also have abnormalities in REM sleep latency, dexamethasone suppression of plasma cortisol and other characteristics like those found in affective disorder patients. Patients with menstrual cycle-related psychopathology and patients with mild presenile dementia are beginning to be studied in a similar comparative fashion across psychological, biological and familial dimensions.

Antidepressant Drug Effects in Different Diagnostic Groups. Of special interest are treatment responses in patients with affective disorders compared to other disorders with possible common features. Because of our extensive background with the MAO-inhibiting antidepressant, clorgyline, and because this drug appears to have a more well-defined mode of action than other antidepressants, its efficacy in the patients with obsessive-compulsive disorder was compared with that of the tricyclic antidepressant, clomipramine. While clorgyline had proved effective in severe primary affective disorder patients with poor responses to other antidepressants, it was ineffective in the obsessive-compulsive patients. Clomipramine had significant effects although, in contrast to our earlier studies in depressed patients, tricyclic responders were not differentiated from non-responders by their pretrial activation or antidepressant responses to single doses of d-amphetamine. The questions involved in these studies were examined at a symposium sponsored by the Branch at last year's American College of Neuropsychopharmacology meeting entitled "Therapeutic Responses to Tricyclic Antidepressant Drugs in Non-Affective Disorder Patients."

Noradrenergic Neurotransmitter Changes with Antidepressant Drug Treatment. Another phase of our studies with affective disorder patients is the continued evaluation of the extensive data collected from our work comparing the selective monoamine oxidase type A inhibitor, clorgyline, with a partially selective MAO-B inhibitor, pargyline, and, more recently, with the more selective inhibitor of MAO-B, α -deprenyl. The small α -deprenyl study was initiated because of new evidence of its possible antidepressant efficacy in certain patient populations, and because it has been reported to not possess the side-effect of tyramine-related hypertensive crises. Data from the α -deprenyl study have not yet been analyzed, but our preliminary conclusions from the clorgyline study which suggested an association between norepinephrine and norepinephrine metabolite changes and clinical antidepressant efficacy has been strengthened by some careful analysis of the cardiovascular effects of clorgyline patients by Dr. Benjamin Roy.

Clorgyline administration was accompanied by a gradual reduction in systolic and diastolic blood pressures and a slowing in pulse during the first two weeks of treatment. During the third and fourth weeks of treatment and the first post-treatment week, mean arterial blood pressure reductions became maximal, together with relatively greater reductions in standing than recumbent blood pressure, i.e. the development of orthostatic hypotension. During the last two weeks of clorgyline administration, pulse rate increases elicited by standing failed to increase further with the greater fall in standing blood pressure occurring at this time, suggesting an alteration in this regulatory mechanism.

Pretreatment systolic and diastolic blood pressures and pulses were significantly correlated with blood pressure reductions in the fourth week of clorgyline treatment as well as with clinical improvement as indicated by reductions in depression ratings on the Hamilton and Beck scales. Reductions in diastolic blood pressure and pulse during clorgyline administration were also significantly correlated with depression rating reductions. Decreases in plasma norepinephrine and in plasma and urine 3-methoxy, 4-hydroxy phenylglycol (MHPG)

concentrations suggested that the blood pressure reductions and clinical antidepressant responses produced by clorgyline were accompanied by reductions in central sympathetic outflow.

These clinical data closely resemble the expected consequences of the changes in brain noradrenergic metabolism and receptor adaptation following clorgyline which suggested a downregulation of central noradrenergic functioning during treatment with this antidepressant. This interpretation is in keeping with the revised hypotheses for depression invoking dysregulation of catecholamine functions and, in one version, postulating an overactive, inefficient state of catecholaminergic synaptic function in endogenously depressed patients. Some direct evidence in favor of this view comes from data published this year with Dr. Ray Lake indicating that our depressed patients as a group have highly significant plasma norepinephrine elevations in comparison to well-matched controls. The functional state of the central noradrenergic system might alternatively be interpreted as being reequilibrated by the changes produced by antidepressant drugs, perhaps establishing a new more beneficial steady state.

Brain Neurotransmitter System Function Assessed in Patients Using Pharmacologic Challenges. Another approach used regularly to evaluate the status of brain neurotransmitter functioning in patients with affective disorders is the pharmacologic challenge strategy. In the past we have used the behavioral, cardiovascular and endocrine responses to agents including amphetamine, L-dopa, dopamine, phenylethylamine, apomorphine, L-tryptophan, physostigmine and arecholine to evaluate possible differences between controls and patient subgroups, before and during antidepressant treatments. The most useful of such probes are generally the agents with the most specific mechanisms of action. Current challenge agents in use include clonidine and fenfluramine, with new studies beginning using scopolamine and a novel serotonin receptor agonist.

Because of its specificity for α_2 -adrenoceptors, the clonidine studies begun by Dr. Larry Siever and carried out in collaboration with Dr. Thomas Uhde and many other coworkers from other groups proved of special interest. Diminished growth hormone responses to clonidine compared to controls were found in a large proportion of depressed patients. Cortisol reductions following clonidine, in contrast, were found in depressed patients but not controls--a difference that seemed partly related to the elevated baseline plasma cortisol concentrations found in some depressed patients. The question of a different set of regulatory influences on cortisol operating in depressed patients seems of relevance to the abnormalities in dexamethasone suppression of plasma cortisol regularly found in some subgroups of depressed patients, but also in some other patient populations. The fact that some of these responses to clonidine--particularly the cardiovascular changes--are altered during clorgyline treatment provides some of the most direct evidence in man of neurotransmitter receptor alterations developing after long-term but not short-term treatment with antidepressant drugs. This is of obvious help in confirming that under ordinary clinical treatment conditions adaptational events like some observed at the molecular and electrophysiological level in animals may occur in man.

Affective Symptoms Associated with the Menstrual Cycle. About 130 subjects have now taken part in a series of investigations initiated by Dr. Jean Hamilton on menstrual cycle-related affective disorders. Portions of the neuroendocrine data from these studies have been presented at several major scientific meetings over the past year.

Human plasma β -endorphin was shown to be relatively stable through the menstrual cycle. In contrast to predictions of an association between premenstrual symptomatology and endogenous opioid withdrawal put forward by Reid and Yen, data presented at the American College of Neuropsychopharmacology, showed that when symptoms were associated with plasma β -endorphin changes, the symptomatology accompanied a rise in β -endorphin rather than a decline. Of course the possibility remains of a central change in opioid physiology. We have therefore arranged to examine cerebrospinal fluid and plasma levels of β -endorphin that were collected simultaneously at two points during the menstrual cycle by our collaborators, Drs. Parry and Gerner.

Another way to clarify the question of opioid changes at the hypothalamic level would be through a pharmacologic challenge, perhaps using naloxone. Further analyses presented at the Psychosomatic Medicine Society meeting began to tease apart the possible dissociation of β -endorphin related premenstrual symptomatology from other putative indices of stress responsivity such as plasma prolactin, cortisol, and urinary 6-hydroxymelatonin changes.

In addition to the work on opioid physiology, another preliminary finding deserves further investigation. With Drs. Sanford Markey and Lee Poth, Dr. Hamilton was able to demonstrate significant group differences in 6-hydroxymelatonin excretion when individuals were separated according to high and low levels of premenstrual symptomatology as rated on the Moos Scale. Melatonin may have an inhibitory effect on ovulation in women; the suggestion of seasonal alterations in melatonin may be pertinent to the findings of variations in both depressive mood and of menstrual cycle length according to time of the year.

Follow-up neuroendocrine and behavioral data was also obtained from a screening survey of normal volunteers who could be separated into relatively high and relatively low premenstrual syndrome groups, and compared with another group seeking help for premenstrual syndrome difficulties. Although we had been concerned that the self-referred clinic population might be biased by self-selection in comparison with a symptomatic group in the community, these groups did not differ on the MMPI. Consistent with another report, however, we found that the symptomatic premenstrual group in the community had significantly more symptoms of atypical depression (increased sleep, appetite, anger, sexuality) than the low premenstrual syndrome group of normals.

A great deal of data from both the clinical and screening studies remains to be analyzed, including items pertinent to life-events and concurrent daily stress ratings, other personality measures, and clinical histories and questionnaires in family and personal psychiatric history.

In other studies, a new time-sampling technique to assess rapid mood changes was piloted with Dr. Richard Lowenstein for future use in menstrual cycle research. Although variations in mood and behavior are prime concerns in psychiatric and psychosomatic research, most assessments have been limited to daily self-reports or observer ratings averaged over the day. But the recognition of diurnal rhythms in physiological and psychological functioning requires methods that better assess rapid fluctuations within the day and across various situations. In the present study, a special experience sampling method was used to examine mood fluctuations and dissociative states in a woman with a diagnosis of multiple personality who reported premenstrual exacerbations of symptomatology.

Psychotropic Drug Effects on Leukocytic Immunologic Functions. Among blood cells, platelets have been frequently used as readily available cells to examine certain functions of neuropharmacologic interest, including serotonin transport, receptor binding of drugs (e.g. imipramine, clonidine) and enzyme activities (e.g. adenylate cyclase, monoamine oxidase). The platelet model, however, does not incorporate an important feature of neuronal physiology, that of cell-cell communication and information transfer. The leukocyte immune system offers a discrete cell system which relies upon soluble peptide signals for regulation of receptor phenotype and transfer of information between different cell types. Furthermore, these peptide signals are under strict and well defined genetic control.

Dr. Benjamin Roy, in collaboration with several investigators in NINCDS (Dr. Henry McFarland, Dr. Jeffrey Greenstein, Dr. John Rose) has initiated a series of studies investigating the effects of different groups of psychotropic agents including neuroleptics, antidepressants and lithium on immune regulatory mechanisms in leukocytes. Their initial results indicate that a prototypical neuroleptic, chlorpromazine, alters immune functions. Chlorpromazine suppressed production of these peptide signals and induction of their receptors. Drugs like metoclopramide (which share the ability of antipsychotic medications to block dopamine receptors and induce extrapyramidal symptoms, but have no antipsychotic effect) were ineffective. Furthermore, it appears that chlorpromazine differs from another class of neuroleptics represented by haloperidol in its effects on immune function. Chlorpromazine inhibits PHA (phytohemagglutinin)-induced lymphocyte proliferation, interleukin-2 (IL-2 or T cell growth factor) and TAC antigen (the IL-2 receptor) expression as well as OKT3-induced mitogenesis. Haloperidol, on the other hand, has negligible effects on PHA-induced proliferation but inhibits OKT3 mitogenesis. These results suggest that antipsychotic medications are able to alter receptor phenotype by impairment of either the production or activation of specific peptide signals which regulate receptor phenotype expression and sensitivity and that this action is independent of their blockade of dopamine receptors.

These findings are of interest for several reasons. Oligodendrocytes within the brain possess the OKT3 antigen and may be a central nervous system corollary to the suppressor T cell. Measles virus preferentially infects T cells and like chlorpromazine reduces PHA-induced proliferation and blocks IL-2 production. Thus, these findings lend insight into basic immune function and may be pertinent to several major disease processes.

Additional Studies. Other minor projects, case reports and reviews dealing with varied aspects of the assessment and, in particular, the pharmacologic treatment of patients with affective disorders, anxiety, and personality disorders were published last year. Most of these papers were in areas covered extensively in previous annual reports, including several dealing with monoamine oxidase inhibitors and another series on the strategies for assessing neurotransmitter receptor function in man. One case report of special interest described the apparent precipitation by clomipramine of symptoms resembling the "serotonin syndrome," a cluster of neurobehavioral abnormalities frequently observed in rodents following high doses of serotonin precursors.

Significance to Biomedical Research and the Program of the Institute:

These studies have helped affirm noradrenergic neurotransmitter system involvement in depression using new data from additional vantage points. The nature and time course of cardiovascular system changes during antidepressant drug treatment both observed naturalistically and following pharmacologic challenges with clonidine parallel closely those found in greater cellular and molecular detail in animals, and thus have helped clarify at least one important aspect of the actions of antidepressant drugs. Other data is helping to broaden our views regarding the discreteness of psychiatric diagnoses and the specificity of "antidepressant" drugs.

Proposed Course:

We are planning a set of studies complimentary to the ones accomplished with clonidine, using a battery of pharmacologic challenge agents with serotonergic system specificity. There is special interest in this neurotransmitter system in relation to the affective disorders, and in addition linkages to plausible hypotheses regarding obsessive-compulsive disorder and premenstrual symptomatology. We have good rodent and non-human primate models to work from, and access to assay approaches to perform these comparative studies in controls and in patients prior to and during antidepressant treatment.

Publications:

Campbell, I.C., Marangos, P.J., Parma, A., Garrick, N.A., and Murphy, D.L.: Localization of monoamine oxidases A and B in primate brains relative to neuron-specific and non-neuronal enolases. Neurochem. Res. 7: 657-666, 1982.

Cohen, R.M., Pickar, D., Garnett, D., Lipper, S., Gillin, J.C., and Murphy, D.L.: REM sleep suppression induced by selective monoamine oxidase inhibitors. Psychopharmacology 78: 137-140, 1982.

Copeland, E.S., Campbell, I.C., and Murphy, D.L.: Interaction between cytosolic monoamine oxidase and spin-labeled amphetamine and its modification by clorgyline and pargyline. Biochim. Biophys. Acta 743: 186-194, 1983.

Garrick, N.A., and Murphy, D.L.: Monoamine oxidase type A: Differences in selectivity towards α -norepinephrine compared to serotonin. Biochem. Pharmacol. 31: 4061-4066, 1982.

Siever, L.J., Uhde, T.W., Lake, C.R., Jimerson, D.C., Risch, S.C., and Murphy, D.L.: Evaluations of alpha-adrenergic responsiveness to clonidine challenge and noradrenergic metabolism in the affective disorders and their treatment. Psychopharmacol. Bull., 8: 118-119, 1982.

Siever, L.J., Uhde, T.W., Potter, W.Z., and Murphy, D.L.: Norepinephrine in the affective disorders. II. Receptor assessment strategies. In Lake, C.R., and Ziegler, M.G. (Eds.): Norepinephrine: Clinical Aspects. Baltimore, Williams and Wilkins Co., in press.

Coursey, R.D., Buchsbaum, M.S., and Murphy, D.L.: Monoamine oxidase activity and the longitudinal biological high risk approach to schizophrenia and affective illnesses. In Mednick, S.A., and Harway, M. (Eds.): Longitudinal Research in the United States. Boston, Martinus Nijhoff, in press.

Guttmacher, L.B., Murphy, D.L., and Insel, T.R.: Pharmacologic models of anxiety. Compr. Psychiatry, in press.

Insel, T.R., and Murphy, D.L.: Pharmacologic response and subgroups of patients with affective illness. In Post, R.M., and Ballenger, J.C. (eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins Co., in press.

Murphy, D.L., Cohen, R.M., Garrick, N.A., Siever, L.J., and Campbell, I.C.: Utilization of substrate selective monoamine oxidase inhibitors to explore neurotransmitter hypotheses of the affective disorders. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins Co., in press.

Murphy, D.L., Garrick, N.A., and Cohen, R.M.: Monoamine oxidase inhibitors and monoamine oxidase: Biochemical and physiological aspects relevant to human psychopharmacology. In Burrows, G.D., Norman, T.R., and Davies, E. (Eds.): Drugs in Psychiatry - Volume I - Antidepressants. Amsterdam, Elsevier/North-Holland Biomedical Press, 1983, Chapter 15, pp. 209-227.

Murphy, D.L., Guttmacher, L.B., and Cohen, R.M.: Recent developments regarding the use of monoamine oxidase inhibitors in psychopharmacology. In Stancer, H.C., Rakoff, Z.M., and Garfinkel, P.E. (Eds.): Guidelines for the Use of Psychotropic Drugs. New York, Spectrum Press, in press.

Risch, S.C., Janowsky, D.S., Parker, D., Kalin, N.H., Aloï, J.A., Cohen, R.M., and Murphy, D.L.: Neuroendocrine abnormalities in depression: Possible cholinergic mechanisms. In Post, R.M., and Ballenger, J.C. (eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins Co., in press.

Siever, L.J., Guttmacher, L.B., and Murphy, D.L.: Serotonergic receptors: Evaluation of their possible role in the affective disorders. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins Co., in press.

Lake, C.R., Pickar, D., Ziegler, M.G., Lipper, S., Slater, S., and Murphy, D.L.: High plasma norepinephrine in patients with major affective disorders. Am. J. Psychiatry 139: 1315-1318, 1982.

Murphy, D.L., Cohen, R.M., Siever, L.J., Roy, B., Karoum, F., Wyatt, R.J., Garrick, N.A., and Linnoila, M.: Clinical and laboratory studies with selective monoamine-oxidase-inhibiting drugs: Implications for hypothesized neurotransmitter changes associated with depression and antidepressant drug effects. In Beckmann, H., and Riederer, P. (Eds.): Monoamine Oxidase and Its Selective Inhibitors: New Concepts in Therapy and Research. Basel, Karger, 1983, pp. 287-303.

Murphy, D.L., Siever, L.J., Cohen, R.M., Roy, B.F., and Pickar, D.: Slowly-developing biological changes during longer-term antidepressant drug treatment: Some clinical evidence supporting the possible involvement of neurotransmitter receptor sensitivity changes in man in the mode of action of antidepressant drugs. In Davis, J.M., and Maas, J.W. (Eds.): The Affective Disorders. Washington, D.C., American Psychiatric Press, 1983, Chap. 23, pp. 317-332.

Risch, S.C., Janowsky, D.J., Kalin, N.H., Cohen, R.M., and Murphy, D.L.: Cholinergic-endogenous opioid mechanisms in mood and cognition in man. Psychopharmacol. Bull., in press.

Siever, L.J., Uhde, T.W., Insel, T.R., Roy, B.F., and Murphy, D.L.: Growth hormone response to clonidine unchanged by chronic clorgyline treatment. Psychiatry Res. 7: 139-143, 1982.

Zuckerman, M., Ballenger, J.C., Jimerson, D.C., Murphy, D.L., and Post, R.M.: A correlation test in humans of the biological models of sensation seeking impulsivity, and anxiety. In Zuckerman, M. (Ed.): Biological Bases of Sensation Seeking, Impulsivity, and Anxiety. Hillsdale, NJ: Lawrence Erlbaum Assoc., 1983, pp. 230-248.

Siever, L.J., Pickar, D., Lake, C.R., Cohen, R.M., and Murphy, D.L.: Extreme elevations in plasma norepinephrine associated with decreased α -adrenergic responsivity in major depressive disorder: Two case reports. J. Clin. Psychopharmacol. 3: 39-41, 1983.

Siever, L.J., Uhde, T.W., and Murphy, D.L.: Possible subsensitization of α_2 -adrenergic receptors by chronic monoamine oxidase inhibitor treatment in psychiatric patients. Psychiatry Res. 6: 293-302, 1982.

Donnelly, E. F., Goodwin, F.K., Murphy, D.L., and Waldman, I.N.: Intellectual function in primary affective disorder. Br. J. Psychiatry 140: 633-636, 1982.

Siever, L.J.: Genetic factors in borderline personalities. In Grinspoon, L. (Eds.): American Psychiatric Review. Washington, D.C., American Psychiatric Press, 1982, pp. 437-456.

Siever, L.J., Insel, T.R., Uhde, T.W., and Murphy, D.L.: Biogenetic factors in the personality disorders. In Frosch, J.P. (Ed.): Current Perspectives on Personality Disorders, in press.

Siever, L.J., Jimerson, D.C., Uhde, T.W., Kafka, M.S., Lake, C.R., Targum, S., and Murphy, D.L.: Clinical studies of monoamine receptors in the affective disorders and receptor changes with antidepressant treatment. Progress in Neuropsychopharmacology and Biological Psychiatry, in press.

Siever, L.J., Kafka, M., Insel, T., Lake, C.R., and Murphy, D.L.: Effect of long-term clorgyline administration on human platelet alpha-adrenergic receptor binding and platelet cycle AMP responses. Psychiatry Res., in press.

Siever, L.J., and Uhde, T.W.: New studies and perspectives on the noradrenergic receptor system in depression: Effects of the alpha-adrenergic agonist clonidine. Biol. Psychiatry, in press.

Siever, L.J., Uhde, T.W., and Murphy, D.L.: Strategies for assessment of noradrenergic receptor function in patients with affective disorders. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders. Baltimore, Williams and Wilkins Co., in press.

Hamilton, J.A., Aloï, J., Mucciardi, B., and Murphy, D.L.: Human plasma β -endorphin through the menstrual cycle. Psychopharmacol. Bull., in press.

Hamilton, J.A., and Parry, B.: Sex-related differences in clinical drug response: Implications for women's health. J. Am. Med. Women's Assoc., in press.

Hamilton, J.A., Haier, R.J., and Buchsbaum, M.S.: Intrinsic enjoyment and boredom coping scales: Validation with personality, evoked potential and attention measures. J. Personality and Individual Differences, in press.

Hamilton, J.A.: Development of interest and enjoyment in adolescence: Attentional capacities (Part I). J. Youth and Adolescence, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00329-08 CN
PERIOD COVERED <div style="text-align: center; font-size: 1.2em;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> <u>Platelets and other systems as models for the study of neurotransmitter function</u>		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Jonathan L. Costa Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS <i>(if any)</i> MTB, NIDR; LPD, SCM, LPD, NIAID; LTB, NCI; CPB, NHLBI; LC, NIADDK; ETB, NINCDS; Lab. de Biochimie, Hôpital St.-Louis, Paris; Dept. of Biochemistry, Univ. of Stockholm		
LAB/BRANCH <u>Clinical Neuropharmacology Branch</u>		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center; font-size: 1.2em;">3.0</div>	PROFESSIONAL: <div style="text-align: center; font-size: 1.2em;">2.5</div>	OTHER: <div style="text-align: center; font-size: 1.2em;">0.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <div style="text-align: justify;"> <p>A number of model systems have been used to extend our understanding of amine metabolism, storage, and transport, and of the mechanism of action of <u>tricyclic antidepressants</u>. Systems studied include <u>liposomes</u>, <u>mitochondria</u>, <u>eukaryotic cells</u>, <u>microvessels</u>, <u>nerve microsacs</u>, and <u>platelets</u>. In addition to their effects on amine uptake, <u>tricyclic antidepressants</u> alter many aspects of energy metabolism in both mammalian mitochondria and eukaryotic cells of fungi and protozoa. Vesicular amine storage may be mediated by the relative impermeability of the vesicle membrane to stored amines, and psychoactive substances known to cause amine release may act in part by increasing the membrane permeability. Both vesicular storage and amine uptake across the plasma membrane may be influenced by membrane lipid fluidity, and the action of some uptake inhibitors appears to be modulated by similar parameters.</p> </div>		

Other collaborative professional personnel engaged on the project:

E. D. Eanes	Section Chief	MTB NIDR
E. C. Weinbach	Section Chief	LPD NIAID
K. J. Kwon-Chung	Section Chief	SCM NIAID
R. Blumenthal	Section Chief	LTB NCI
D. Silberstein	Guest Worker	LPD NIAID
A. Robinson	Guest Worker	CPB NHLBI
C. R. Creveling	Biochemist	LC NIADDK
K. L. Kirk	Chemist	LC NIADDK
S. J. Morris	Expert Consult	ETB NINCDS
J.-M. Launay	Asst. des Hospitaux	Lab. de Biochimie, Hôpital St.-Louis, Paris, France
L. Ernster	Prof. of Chemistry	Univ. of Stockholm
B. D. Nelson	Prof. of Chemistry	Univ. of Stockholm

Project Description:

Objectives: To explore mechanisms for the disposition of biogenic amines by tissues adapted for amine uptake, storage, and metabolism. Although human platelets are the principal cell types examined, capillaries (microvessels) and nerve ending preparations (microsacs) are also studied. In addition, hypotheses concerning the mechanism of action of drugs known to alter amine disposition in neural tissue are tested by examination of their effects in various non-neural tissue preparations (e.g., fungi, protozoa, and mammalian mitochondria).

Studies Implemented: (1) Evaluation of the mechanism of ionophore-mediated transport of calcium into liposomes, and of the effects of various drugs on the transport process; (2) examination of action of inhibitors of dopamine uptake and tricyclic antidepressants on energy metabolism, electron transport, proton gradients, and metabolite transport in mammalian mitochondria and other eukaryotic cells (fungi, protozoa); (3) exploration of the possibility that vesicular amine storage sites are present in microvessels; (4) assessment of factors enhancing or retarding the outflow of dopamine from nerve microsacs; (5) investigations of the action on platelets of various compounds which may inhibit phenolsulfotransferase activity; (6) assessment of the permeability properties of platelet plasma and vesicular membranes, with special reference to the uptake and storage of serotonin.

Methods Employed:

1. General Preparative Procedures: Platelets were isolated from normal volunteers by differential centrifugation or by plateletpheresis. Dense bodies (amine storage vesicles) were isolated utilizing Metrizamide gradients. Liposomes were formed by reversed-phase evaporation or by sonication of appropriate solutions. Mitochondria were prepared by homogenization and differential centrifugation, and sub-mitochondrial particles and isolated ATPases were prepared by extraction, column chromatography and centrifugation. Fungi and protozoa were grown from pure or axenic cultures supplied by the American Type Culture Collection. Microvessels were isolated from fat pads, brains, and hearts of rats and guinea pigs by centrifugation over Percoll gradients, and microsacs by centrifugation and filtration.

2. Evaluation of Calcium Transport in Liposomes. Multilamellar liposomes which were positively charged, negatively charged and neutral were formed so as to encapsulate solutions of defined ionic composition, and the kinetics of calcium accumulation was studied in the presence and absence of ionophores and various drugs.

3. Effects of Tricyclic Antidepressants on Cellular Function. Oxygen consumption and spectral (absorbance) changes, associated with oxidation or ATPase activity were monitored in intact mitochondria, submitochondrial particles, isolated F_1 -ATPase, or chromaffin granules. Growth, transport of sugar and proline, and trans-membrane proton gradients were measured in protozoal and fungal cells, both under control conditions and in the presence of tricyclic antidepressants and inhibitors of dopamine uptake.

4. Amine Uptake and Storage in Microvessels and Microsacs. In microvessels, attempts to verify the existence of amine storage sites were made by examining the uptake and subsequent loss of unlabelled quinacrine and radiolabelled serotonin in the presence and absence of agents known to interfere with vesicular storage. In microsacs, peptides and anion transport inhibitors were used to perturb the uptake, metabolism, and efflux of endogenous dopamine as quantitated by high-pressure liquid chromatography.

5. Evaluation of Possible Phenolsulfotransferase Inhibitors. Various compounds synthesized chemically were incubated with human platelets and the activity of phenolsulfotransferase was assayed both in intact platelets (by measuring the extent of O-sulfation of radiolabelled serotonin), and in cell preparations disrupted by sulfhydryl-activated bacterial toxins. Vesicular uptake, storage, and release of radiolabelled serotonin was also measured.

6. Permeability Properties of Platelet Membranes. Amine storage vesicles in intact human platelets and following isolation were loaded with radiolabelled serotonin or unlabelled quinacrine. Permeability of the vesicle membrane was studied in the presence of an osmotic gradient (water permeability), quinacrine, tyramine, and psychoactive substances (fluphenzine, fenfluramine). The uptake of labelled serotonin across the platelet plasma membrane was examined following variation of the temperature and the addition of inhibitors of amine uptake.

Major Findings:

1. Calcium Transport in Model Systems. The liposomal membrane of negatively-charged liposomes is sufficiently permeable to calcium to permit its entry into the liposome interior, where it appears to bind to membrane-associated diacetyl phosphate groups. Negatively charged and neutral liposomes fail to accumulate any calcium. Doping of the liposomal membrane with a calcium ionophore greatly accelerates the rate of calcium entry, provided other ionic counter-gradients exist. Drugs which are believed to block calcium channels in excitable tissue appear to inhibit the ionophore-mediated movement of calcium.

2. Effects of Tricyclic Antidepressants on Cellular Function. Tricyclic antidepressants and some inhibitors of dopamine uptake can act at micromolar concentrations to uncouple mitochondrial oxidative phosphorylation. In mitochondria and submitochondrial particles, these compounds do not exhibit the protonophoric activity associated with classical uncouplers, and their effects on ATPase activities and electron transport are also inconsistent with the behavior of other uncoupling agents. Although the tricyclic antidepressants appear to be fairly specific inhibitors of mitochondrial-linked ATPase, they inhibit the ATPase of isolated chromaffin granules and chromaffin granule ghosts only at much higher concentrations. In addition, the compounds appear to act as protonophores (i.e., to collapse pH gradients and to interfere with sugar and amino acid transport) in fungi and protozoa, eukaryotic cells which maintain a pH gradient across their plasma membranes.

3. Amine Uptake and Storage in Microvessels and Microsacs. Microvessels examined by electron microscopy appear to have no vesicular storage sites for amines. Nevertheless, our data suggests that both endogenous and newly-acquired

serotonin are sequestered in a reserpine-sensitive compartment in which the serotonin is not accessible to monoamine oxidase. Nerve microsacs prepared from guinea pig striata metabolize and exteriorize endogenous dopamine stores at a similar rate and to a similar extent in the presence of several compounds which are potent inhibitors of the transport of organic acids across cell membranes. It thus seems unlikely that acidic dopamine metabolites are extruded from the cell by an active-transport type of mechanism.

4. Evaluation of Possible Phenolsulfotransferase Inhibitors. Initial studies suggested that the phenolsulfotransferase activity in human platelets had a unique substrate range and specificity, and subsequent work has substantiated and extended this concept. Ring fluorination of either serotonin or tyramine enhances the apparent maximal velocity of sulfation by factors of 3 to more than 20, while the apparent enzyme-substrate affinity constant remains unchanged or is only slightly decreased. In addition, there appear to be two separate sites of enzyme activity for several substrates, including dopamine and normetanephrine. Many compounds which are inhibitory for phenolsulfotransferase in other tissues are substrates for the enzyme in human platelets; nevertheless a recently-synthesized substituted nitrostyrene compound produces up to 80% inhibition of the platelet enzyme and up to 90% inhibition of the enzyme in rat brain.

5. Permeability Properties of Platelet Membranes. (a) Storage vesicle membranes: The membranes surrounding the amine storage vesicles (dense bodies) in human platelets appear to have unusual permeability properties, whose control and alteration may play an important role in the maintenance and release of stored amines. Unlike most cellular and organelle membranes, the dense body membrane appears to be essentially impermeable to water, since dense bodies inside intact platelets and following isolation do not swell or lyse during exposure to extremely hypo-osmolar solutions. Movement of serotonin by an active transport process across the membrane into the interior of the dense body is quite rapid in intact platelets. Nevertheless, removal of the dense body from its intracellular milieu results in a 50-fold decrease in transport velocity, possibly because some sort of close association between the plasma and vesicular membranes is required for serotonin uptake to proceed at a rapid rate. Once serotonin is present at high concentrations inside the dense body, its retention appears to be mediated to a large extent by the relative impermeability of the membrane to passive serotonin fluxes. The lipid composition and fluidity of the membrane may play an important role, since increasing the temperature permits both an increased efflux of serotonin and an increased entry of the lipophilic amine quinacrine. Furthermore, several membrane-active agents which cause serotonin release at 37°C fail to do so at 0°C, despite their ability to enter the vesicular interior at 0°C. One can postulate that many "releasing agents" may act to cause loss of vesicular serotonin not because of displacement from intra-vesicular binding sites, but rather because they increase the passive permeability of the membrane to serotonin.

(b) Plasma membranes: The plasma-membrane uptake of serotonin, as well as the action of uptake-inhibiting drugs, may also be influenced by lipid composition and fluidity, since temperature can have a profound effect on the velocity processes. As the temperature is decreased below 37°C, the maximal uptake decreases, but the affinity constant remains the same. Concomitantly, the

concentration of a number of compounds required to inhibit serotonin uptake by 50% increases (in many cases by an order of magnitude for a 10°C decrease in temperature). Although the substances tested have been reported to act by similar mechanisms (i.e. non-competitive binding at a site closely associated with the serotonin carrier), the extent and nature of the inhibition as a function of temperature varies widely. In addition, the IC_{50} for imipramine does not change as a function of temperature, in contrast to that for chlorimipramine.

Significance to Biomedical Research and the Program of the Institute:

The work continues to refine our understanding of the mechanisms responsible for the uptake and storage of amines in platelets and microvessels. Attempts to develop useful inhibitors of phenosulfotransferase may assist in elucidation of the role of this enzyme in amine metabolism in normal and pathological states. Evaluation of the actions of tricyclic antidepressants has demonstrated that these drugs can affect energy metabolism at several sites, and in organisms from many different phyla. Continued exploration of the mechanisms responsible for these actions should extend our understanding of the relationship between energy metabolism, amine uptake, and the psychoactive effects of these compounds.

Proposed Course:

Continue the use of platelets, microvessels, and nerve microsacs as tools to study aminergic function. Areas to be emphasized are (1) the interaction of various drugs with phenosulfotransferase, (2) exploration of the functional significance of phenosulfotransferase, (3) relationship of lipid fluidity to platelet amine uptake and storage, and (4) interaction of psychoactive drugs with energy metabolism.

Publications:

Fay, D.D., Costa, J.L., and Launay, J.-M.: Thin layer chromatographic separation and quantitation of radioactively-labelled 5-hydroxytryptamine, 5-hydroxytryptamine-O-sulfate, and 5-hydroxyindoleacetic acid. J. Chromatogr. 252: 338-341, 1982.

Costa, J.L.: Mitochondries et plaquettes: modeles pour l'etude des substances actives sur le systeme nerveux. Act. Pharm. Biol. Clin. 2: 85-98, 1983.

Eanes, E.D., and Costa, J.L.: X-537A ionophore-mediated calcium transport and calcium phosphate formation in Pressman cells. Calcif. Tissue Int. 35: 250-257, 1983.

Costa, J.L., Launay, J.-M., and Kirk, K.L.: Exploration of the role of phenosulfotransferase in the disposition of serotonin in human platelets: implications for a novel therapeutic strategy against depression. Med. Hypotheses 10: 231-246, 1983.

Costa, J.L., and Fay, D.D.: Effects of media of low osmolarity on the dense bodies of human platelets. Biochem. Pharmacol. 32: 1365-1369, 1983.

Weinbach, E.C., Costa, J.L., Claggett, C.E., Fay, D.D., and Hundal, T.: Reserpine as an uncoupler of oxidative phosphorylation and the relevance to its psychoactive properties. Biochem. Pharmacol. 32: 1371-1377, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00330-05 CN
PERIOD COVERED <div style="text-align: center;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Use of electron and photon imaging techniques to study aminergic systems		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) John L. Costa Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) LC, NIADDK; LAS, DCRT; MAS, BEIB; Geo. Wash. Univ.; IBM Lab; SUNY Stonybrook; Brookhaven Natl. Lab; Naval Res. Lab; Univ. of Illinois; Wash. State Univ.; Maxwell Labs; Lawrence Livermore Lab; Los Alamos Lab		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">2.5</div>	PROFESSIONAL: <div style="text-align: center;">2.0</div>	OTHER: <div style="text-align: center;">0.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Electron microprobe analysis</u> has been employed to examine the phosphorus and divalent cations in the storage granules in <u>bacteria</u> and in <u>platelets</u> of cows with storage pool deficiency. <u>Mass thickness measurements</u> with a scanning-transmission electron microscope have confirmed the localization of iodinated quinacrine in <u>platelet dense bodies</u>, and should prove useful in the subcellular localization of other iodinated tracers as well. <u>Contact x-ray microscopy</u> of dried specimens has revealed novel structure in platelets, nervous tissue, and bacteria, and flash <u>x-ray microscopy</u> of hydrated specimens has been accomplished. Preliminary work indicates that <u>x-ray holography</u> of similar specimens will be possible. </p>		

Other collaborative professional personnel engaged on the project:

K. L. Kirk	Chemist	LC NIADDK
C. R. Creveling	Biochemist	LC NIADDK
J. J. Bailey	Section Chief	LAS DCRT
M. Douglas	Computer Analyst	LAS DCRT
R. Leapman	Physicist	MAS BEIB
J. Webster	Postdoctoral Fellow	Geo. Wash. Univ.
R. Feder	Staff Scientist	IBM Lab
D. Sayre	Staff Scientist	IBM Lab
J. Kirz	Professor	SUNY Stonybrook
J. Wall	Staff Scientist	Brookhaven Natl. Lab
J. Hainfield	Staff Scientist	Brookhaven Natl. Lab
J. Nagel	Staff Scientist	Naval Res. Lab
C. Rhodes	Professor	Univ. of Illinois
K. Meyers	Assoc. Professor	Wash. State Univ.
J. Pearlman	Manager	Maxwell Labs
G. Chapline	Staff Scientist	Lawrence Livermore Lab
J. Solem	Staff Scientist	Los Alamos Lab
S. Layne	Staff Scientist	Los Alamos Lab

Project Description:

Objectives: To develop and utilize imaging techniques which reveal cellular ultrastructure in conjunction with the elemental composition of discrete regions. The imaging particles include both electrons and photons, and specimens are examined in both dried and fully hydrated states.

Studies Implemented: (1) Use of mass thickness measurements in a scanning-transmission electron microscope to detect iodine-labelled quinacrine in human platelets; (2) electron microscopy and microprobe analysis of platelets from normal cows and cows with 'storage pool deficiency (the Chediak-Higashi syndrome); (3) electron microscopy and microprobe analysis of bacteria containing poly-phosphate granules; (4) use of the long-wavelength (soft) x-ray beam line at Brookhaven for studies of near edge fine structure of model compounds, and for contact x-ray microscopy of human platelets utilizing defined wavelengths; (5) contact x-ray microscopy of dried blood cells, nervous tissue, and bacteria utilizing an electron-gun source; (6) contact x-ray microscopy of hydrated cells utilizing a pulsed-plasma x-ray source; (7) experiments preparatory for x-ray holography of blood cells (to be carried out utilizing an x-ray laser).

Methods Employed:

1. General Preparative Procedures: Platelets and red blood cells were prepared by differential centrifugation of whole blood, nerve microsacs by homogenization and filtration of guinea pig cortices, and bacteria by growth on agar plates. Whole mounts were prepared by rapid air drying or by fixation and critical-point drying; thin sections were cut from material fixed, dehydrated, and embedded in Epon. Dense bodies were isolated from human platelets by sonication, differential centrifugation, and centrifugation over Metrizamide density gradients.

2. Scanning Transmission Electron Microscopy. Air-dried whole mounts of platelets or isolated dense bodies were examined in a scanning-transmission electron microscope equipped with electron detectors for quantitating elastic electron scattering (mass thickness). Images were recorded digitally and processed on a D'Anza computer graphics display and analysis system. Special programs were developed for defining the perimeter of a dense body and for calculating the average mass thickness of the entire dense body.

3. Electron Microscopy and Microprobe Analysis. Whole mounts and thin sections were studied and photographed in a transmission electron microscope equipped with a scanning attachment and an x-ray detector and analysis system.

4. Soft X-Ray Beam Line Work. Very thin layers (approximately 1-2 μm thick) of model compounds were prepared by rapid evaporation of solution onto thin substrates of poly-methyl-methacrylate. Near-edge structure was defined by transmission spectroscopy of long-wavelength (soft) x-rays through the specimens, utilizing a specially-equipped beam line at the Brookhaven Synchrotron. Contact x-ray images of human platelets were obtained by exposing platelet-resist sandwiches with x-rays of defined wavelength.

5. Contact X-Ray Microscopy. Contact x-ray images of whole mounts on thin sections were produced by allowing soft x-rays from an electron gun to pass through the specimen and impinge on a resist composed of co-polymer or poly-methyl-methacrylate. The exposed resist was developed in organic solvents, coated with a thin layer of metal, and studied either in a transmission or a scanning electron microscope.

6. Pulsed-Plasma X-Ray Microscopy. Hydrated blood cells and bacteria were sequestered in a thin (1-5 μm) layer of buffer by placement in a well system micro-fabricated from silicon nitride and poly-methyl-methacrylate. The entire assembly was mounted in a vacuum-sealed holder and exposed to a 100-nanosecond burst of soft x-rays, produced by a gas-jet pulsed plasma x-ray source. Developed resists were coated with metal and studied in a scanning electron microscope.

7. X-Ray Holography. In preparation for production of x-ray holograms of biological specimens, specimen holders were designed, and decisions were made concerning which specimens to examine. To explore the reciprocity characteristics of resist, as well as the stability of biological specimens irradiated with intense ultra-short x-ray pulses, exposures of platelets were made utilizing sources producing nanosecond or picosecond pulses of far-ultraviolet radiation or soft x-rays.

Major Findings:

1. Mass Thickness of Platelet Dense Bodies. The absolute mass thickness of the platelet dense body core is not correlated with its diameter. This finding suggests first that the total amount of calcium and phosphates comprising the dense body core achieves a relatively constant concentration regardless of the surface area available for accumulation, and is consistent with other observations that the core exists in the form of an amorphous solid. Second, it permits detection of differences of at least 20% in the average mass thickness of two populations of dense bodies with relatively small sample sizes. Comparison of the mass thicknesses of dense bodies inside platelets and following isolation indicates that the two populations have similar densities, precluding large-scale loss of core constituents during the isolation process. Incubation of platelets with iodoquine (the ring-iodinated analogue of quinacrine) increases the mass thickness of dense bodies by approximately 25%, an increment consistent with previous data suggesting quinacrine accumulation in dense bodies.

2. Platelets from Chediak-Higashi Cows. Platelets from normal cows appear to contain two types of serotonin storage sites, both of which are electron-opaque and contain phosphorus. One type is rich in calcium and contains some magnesium, while the other sequesters much lower concentrations of divalent cations. Platelets from Chediak-Higashi cows contain very few calcium-rich dense bodies, but normal numbers of the second type of dense body structures, which may be responsible for the thrombin-releasable calcium and magnesium found in these platelets.

3. Bacterial Polyphosphate Granules. Under certain growth conditions, many species of bacteria form cytoplasmic granules which are rich in polyphosphate and do not have a limiting membrane. If divalent cations are available in

the medium, they will be incorporated into the granule structure, but may be displaced when the granules accumulate polyamines.

4. Synchrotron Studies of Model Compounds and Platelets. One useful approach to holographic x-ray imaging of biological specimens is to utilize illuminating x-rays with a wavelength which is strongly absorbed by nitrogen. Improved image quality can be obtained by tuning the x-ray laser to the wavelengths absorbed most strongly by the nitrogen in proteins or nucleic acids. The positions of the nitrogen absorption edges in thin films of albumin or nucleic-acid bases have been defined utilizing Synchrotron radiation. Contact x-ray micrographs of human platelets have also been obtained with Synchrotron radiation, utilizing x-ray wavelengths which bracket the calcium and oxygen absorption edges. Appropriate processing of the two exposures should produce images which highlight calcium- or oxygen-rich features.

5. Contact X-Ray Microscopy (Electron Gun Source). Contact x-ray microscopy of a number of tissues and cell types continues to reveal structural features which are not visible when the identical preparations are studied by conventional electron microscopy. In thin sections of fixed human platelets, for example, the plasma membrane is underlain by a 50 nm band of photon-absorbent material. Whole mounts of nerve microsacs contain a cytoskeletal network similar to that found in platelets. Many species of bacteria contain "cytoskeletal" type elements whose morphology is highly variable but in general characteristic of the species. This material, which may represent aggregates of bacterial nucleic acid, is intimately associated with polyphosphate granules when they form, and appears to assume a twisted or basket-like configuration in bacteria with a spiral shape (i.e., Spirillum).

6. Contact X-Ray Microscopy of Hydrated Cells (Pulsed Plasma Source). Conditions have been worked out which permit maintenance of blood cells and bacteria in thin layers of water (1-2 μm) for times sufficient to permit exposure in a pulsed plasma x-ray source. Images of hydrated normal red blood cells, sickle red blood cells, bacteria, and human platelets have been obtained, and their ultrastructure is currently being studied.

7. X-Ray Holography. Holographic fringes of model specimens have been obtained with 3 nm soft x-rays, and attempts to reconstruct the holograms are underway. Specimen holders compatible with the configuration of the x-ray laser are being fabricated, and will be tested utilizing pulsed-plasma sources. Contact x-ray of cells can be obtained utilizing a 5 nanosecond pulse of soft x-rays (produced by laser excitation of a titanium carbide target), a finding which suggests that the expected pulse length of 1 nanosecond for the x-ray laser will produce holograms of wet cells without hydrodynamic blurring.

Significance to Biomedical Research and the Program of the Institute:

Electron microprobe analysis continues to provide an extremely useful tool for elemental analysis of amine storing granules, which appear to occur in bacteria as well as in platelets. Mass-thickness measurements with a scanning-transmission electron microscope are useful adjuncts, since this technique has the potential to detect iodine-labelled compounds in platelets and other systems.

X-ray microscopy reveals novel cellular ultrastructure in a variety of neural and non-neural cells and tissues, and continued work on elemental localization should assist in the elucidation of the biological significance of these findings. The method has the particular advantage that it can now image fully hydrated specimens on a nanosecond time scale; the contact technique with slower x-ray sources is important both because of its intrinsic interest and because it will assist in interpreting data obtained on wet cells.

Proposed Course:

Concentrate on obtaining x-ray images of hydrated cells, with special reference to human platelets, and on studies of the elemental composition of human platelets containing fluorinated amines.

Publications:

Feder, R., Pearlman, J.S., Riordan, J.C., and Costa, J.L.: Flash x-ray microscopy of human platelets. J. Microscopy (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00331-05 CN
PERIOD COVERED <div style="text-align: center;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Use of nuclear magnetic resonance to study aminergic systems		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jonathan L. Costa Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) LC, NIADDK; LM NIAAA, LEM, LC, NHLBI; ETB, NINCDS; Johns Hopkins Univ. Wash. State Univ.		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">2.5</div>	PROFESSIONAL: <div style="text-align: center;">2.0</div>	OTHER: <div style="text-align: center;">0.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The technique of <u>nuclear magnetic resonance</u> (NMR) has been used to study the disposition of <u>fluorinated deoxyglucose</u> in intact rats, of 6-fluorodopamine in <u>nerve microsacs</u>, and of <u>difluoroserotonin</u>, <u>carbon-13-labelled serotonin</u>, and <u>lithium in platelets</u> from rabbits, pigs, cows, and humans. <u>Amines inside storage vesicles</u> and in <u>extra-vesicular sites</u> in each tissue type have unique <u>motional properties</u> and <u>chemical-shift parameters</u>, which suggest that each tissue possesses unique storage mechanisms.</p>		

Other collaborative professional personnel engaged on the project:

E. D. Kirk	Chemist	LC NIADDK
C. R. Creveling	Biochemist	LC NIADDK
R. L. Veech	Lab Chief	LM NIAAA
R. Balaban	Staff Fellow	LEM NHLBI
E. A. Sokoloshi	Technican	LC NHLBI
S. J. Morris	Expert Consultant	ETB NINCDS
D. Diffley	Resident in Internal Medicine	Johns Hopkins Univ.
K. M. Meyers	Assoc. Professor	Wash. State Univ.
J. Magnuson	Assoc. Professor	Wash. State Univ.

Project Description:

Objectives: To use nuclear magnetic resonance (NMR) to define in a non-destructive fashion the properties of cellular systems which store biogenic amines, or which sequester nucleotides, and to examine the effects of psychoactive substances (i.e. quinacrine, lithium) on these processes.

Studies Implemented: (1) Characterization of the physical state of nucleotides and pyrophosphate in rat liver mitochondria; (2) exploration of the use of 2-fluoro-2-deoxyglucose in studies of cerebral metabolism; (3) examination of the effects of NMR imaging techniques on drug-induced anesthesia; (4) evaluation of the response of isolated chromaffin vesicles to osmotic dehydration, and its relationship to amine storage; (5) characterization of the physical state of 6-fluorodopamine in storage vesicles and cytoplasm of nerve microsacs; (6) examination of the nature of the intra-vesicular storage complexes for serotonin in platelets of pig, cow, and rabbit; (7) study of the mechanisms of serotonin storage in platelets from cows with storage pool deficiency (the Chediak-Higashi syndrome); (8) more critical definition of the chemical and physical state of C¹³-labelled and fluorinated serotonins inside storage vesicles of human platelets; (9) exploration of the nature of the intra-vesicular storage complex of lithium in human platelets.

Methods Employed:

(1) **General Preparative Procedures.** Intact hepatic parenchymal cells were prepared from rat liver by collagenase digestion, homogenization, and centrifugation through bromodecane. Mitochondria were isolated from the cells following disruption and differential centrifugation through sucrose solutions. Chromaffin vesicles were isolated from homogenized bovine adrenal glands by differential centrifugation. Nerve microsacs were prepared from dissected, homogenized striata of guinea pigs by centrifugation and filtration in isotonic buffers. Platelets were collected from humans by plateletpheresis, and from other species by venipuncture followed by differential centrifugation.

Nerve microsacs or platelets were loaded with lithium, 6-fluorodopamine, difluoroserotonin, or C¹³-labelled serotonin by incubation at 37°C, and prepared for NMR study by resuspension at a high cell density or by extraction with perchloric acid. Chromaffin vesicles were dehydrated by resuspension in hypertonic sucrose solutions, and in some cases loaded with quinacrine by brief incubation at room temperature.

Rats were injected intravenously with 2-fluoro-2-deoxyglucose (2F-2DOG), anesthetized, and placed in the NMR magnet. In some cases, the carotid artery was catheterized and the 2F-2DOG injected immediately before the NMR examination.

(2) **NMR Studies.** Chromaffin vesicles, mitochondria, hepatocytes, nerve microsacs, and platelets were studied as suspensions in glass capillary tubes, and intact rat liver and brain by means of surface coils placed at appropriate positions. Phosphorus-31, fluorine-19, or carbon-13 resonances were observed as a function of temperature, and in some cases T₁'s were estimated utilizing an inversion-recovery sequence of pulses.

Major Findings:

(1) Nucleotides and Pyrophosphate in Rat Liver Mitochondria. When rats are given intra-peritoneal injections of butyrate or acetate, their livers accumulate large quantities of calcium and pyrophosphate, which appear to be sequestered inside the hepatocyte mitochondria. Model studies and topical ^{31}P -NMR of the rat liver in vivo suggests that although intra-mitochondrial nucleotides are complexed to calcium and still free in solution, the pyrophosphate forms an insoluble precipitate with the calcium.

(2) 2F-2-Deoxyglucose and Cerebral Metabolism. Feasibility studies indicate that rats can tolerate intravenous injections of large boluses of 2F-2DOG without convulsions. In addition, the 2F-2DOG can be injected while the rats are in position for ^{19}F -NMR studies. A surface coil suitable for the work has been designed and is being constructed.

(3) NMR and Anesthesia. Preliminary studies suggest that rats anesthetized with pentobarbital wake up when the cranial region is exposed to radio-frequency fields in the NMR magnet. Future experiments will focus on quantitating this effect and exploring the mechanism.

(4) Chromaffin-Granule Dehydration. When isolated chromaffin granules are dehydrated by exposure to hyper-osmotic sucrose solutions, their adenine nucleotides appear to form aggregates of high molecular weight. The aggregates resemble those found in the storage vesicles of pig platelets in that their apparent molecular weight increases as the temperature decreases from 30°C to 4°C. They differ from those in pig platelets, however, in that their apparent molecular weight may be less, and in that they do not decrease their molecular weight when they become associated with the basic amine quinacrine.

(5) 6-Fluorodopamine in Nerve Microsacs. 6-Fluorodopamine inside storage vesicles of nerve microsacs prepared from guinea-pig striata appears to reside in a storage complex unique to this tissue. Unlike fluorodopamines in storage vesicles of human or pig platelets, the compound in nerve microsacs is relatively unrestricted in motion at 4°C. As in human platelets, 6-fluorodopamine in microsacs can also accumulate in an extra-vesicular (cytoplasmic) pool; in this compartment, it appears not to be O-sulfated to any appreciable extent and is unrestricted in motion (i.e., not complexed to adenine nucleotides).

(6) Serotonin Storage in Animal Platelets. As evaluated from the ^{19}F -NMR parameters of difluoroserotonin incorporated into vesicles of rabbit, pig, and cow platelets, each species associates the ring portion of its serotonin with a unique type of storage complex, probably formed by self-association of vesicular adenine nucleotides and divalent cations. The state of aggregation of the storage complex in rabbit platelets changes only slightly as the temperature is lowered to 4°C, while that in pig platelets increases to very high molecular weight at 4°C. These differences may relate to the relative intra-vesicular concentration of magnesium, which is much greater in pig than in rabbit platelets. The resonances of both fluorines in difluoroserotonin in rabbit platelets are shifted at least 1 ppm from those in the parent compound (both free in solution and bound inside pig platelet vesicles). Other compositional differences between the two types of vesicles, perhaps the presence of other biogenic

amines or unusual nucleotides, may account for these phenomena. Cow platelets appear to be unique in that they contain two distinct pools of difluoroserotonin. One pool resembles that found in rabbit platelets, in that at 4°C it permits relatively sharp fluorine resonances with no apparent chemical shift difference (i.e., relatively high molecular correlation times). The other pool is more analogous to that in pig platelets, since at 4°C it exhibits unshifted fluorine resonances which are quite broad.

As examined utilizing the ^{13}C -NMR parameters of serotonin labelled on its side chain with ^{13}C , another aspect of serotonin storage in the platelets of all three species emerges. In all cases, the side chain of intra-vesicular serotonin rotates much more freely than does the indole ring (as estimated from the relative linewidths and the magnetogyric ratios of the two nuclei), and appears to exist in an essentially identical chemical environment in the vesicles of all three species. The data suggest that ionic bonding between the serotonin side chain and nucleotide phosphate groups does not play an important role in the formation and maintenance of intra-vesicular serotonin storage complexes.

(7) Serotonin Storage in Chediak-Higashi Cow Platelets. Platelets from cows with storage pool deficiency (the Chediak-Higashi syndrome) are able to accumulate sufficient difluoroserotonin or ^{13}C -labelled serotonin to produce visible resonances in a high-field NMR instrument. The chemical shift of the fluorine groups corresponds to that seen in only one of the two difluoroserotonin pools found in normal cow platelets. Since dense bodies containing calcium and adenine nucleotides are essentially absent in Chediak-Higashi cow platelets, this resonance has the molecular correlation time associated with a storage site containing magnesium and possibly inorganic phosphate.

(8) Serotonin Storage in Human Platelets. In human platelets, the calcium, nucleotides and pyrophosphate inside amine storage vesicles are in the form of an amorphous solid. When difluoroserotonin is added to platelets, about 90% of the total is sufficiently restricted in motion so that no ^{19}F resonances are visible. The indole ring of this portion of the difluoroserotonin thus appears to become as immobile as if in a solid. Approximately 10% of the total intra-platelet difluoroserotonin produces broad resonances (about 1000 Hz wide), and may represent cytoplasmic amine. If this is the case, extra-vesicular amine in human platelets appears to be either bound or compartmentalized in some fashion, unlike the 6-fluorodopamine in the cytoplasm of nerve microsacs.

Once inside vesicles, the side chain of ^{13}C -labelled serotonin also becomes essentially immobile (i.e., no ^{13}C resonances are visible). The entire serotonin molecule, and not just the indole ring, thus appears to associate closely with the solid core matrix of calcium and phosphate moieties, in contrast to the relative molecular freedom of the serotonin side chain when the indole ring is immobilized in vesicles of platelets from other species. Since resonances from the side chain of extra-vesicular serotonin are of similar width to those of serotonin free in solution, it seems likely that only the indole ring participates in complexation or binding of cytoplasmic amine.

(9) Lithium in Storage Vesicles of Human Platelets. When human platelets accumulate exogenous lithium, more than half of the intracellular lithium enters

the serotonin storage vesicles. Nevertheless, the resonance of intra-vesicular ^7Li is less than 10 Hz wide, and its T1 is similar to that of unbound ^7Li in aqueous solutions. Lithium in human platelet storage vesicles thus appears to exist in free solution, possibly in the aqueous phase, contrast to all other small molecules examined to date. The data are consistent with studies in model systems indicating that lithium does not compete with calcium for binding to the other components of the vesicle core, and suggest that this storage vesicle resembles a bacterial spore, in which most components of small molecular weight exist as a solid phase in an aqueous solution. Since human platelet vesicles appear to contain an aqueous phase capable of solvating cations, stored serotonin molecules must be very tightly bound to the solid core constituents.

Significance to Biomedical Research and the Program of the Institute:

Preliminary work suggests that NMR may be used to study the motional properties and metabolic transformations of fluorinated molecules in living rats. Initial studies may concentrate on 2F-2-deoxyglucose, but further refinement of the apparatus may permit observation of such molecules as 6-fluorodopamine. It also appears as if the NMR process itself may have interesting effects on brain function, a phenomenon which should be explored in more detail. NMR of subcellular preparations and intact cells has characterized the physical and chemical state of amine storage vesicles and extra-vesicular storage mechanisms in chromaffin vesicles, nerve microsacs, and platelets from humans and various animal species. The considerable diversity which exists may relate to the unique secretory requirements for each type of tissue, and suggests that there are differences both in the uptake properties of the vesicle membranes and in the responses of target tissues to substances co-stored with biogenic amines.

Proposed Course:

Explore in more detail the physical state of lithium in the storage vesicles of normal platelets, with the objective of studying in a similar fashion platelets from patients receiving chronic lithium treatment. Continue the use of ring-fluorinated compounds, including quinacrine, to explore storage mechanisms in vesicles and cytoplasm of platelets and nerve microsacs.

Publications:

Costa, J.L., Dobson, C.M., Fay, D.D., Kirk, K.L., Poulsen, F.M., Valeri, C.R., and Vecchione, J.J.: Nuclear magnetic resonance studies of amine storage in pig platelets. FEBS Letters 136: 325-328, 1981.

Costa, J.L., Fay, D.D., Nurnberger, J.I., and Murphy, D.L.: Preferential accumulation of lithium in the dense bodies of human platelets. Biochem. Pharmacol. 31: 3215-3218, 1982.

Diffley, D.M., Costa, J.L., Sokoloski, E.A., Chiueh, C.C., Kirk, K.L., and Creveling, C.R.: Direct observation of 6-fluorodopamine in guinea pig nerve microsacs by ^{19}F NMR. Biochem. Biophys. Res. Commun. 110: 740-745, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00332-05 CN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Animal models for the study of neuropharmacologic effects		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Robert M. Cohen Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) LBC, NIADDK; CN, NSB, NIMH; Maudsley Hospital, London; Howard University		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> A sequential study of the time-dependent effects of the selective type A monoamine oxidase-inhibiting antidepressant <u>clorgyline</u> has begun in rodents. A change in <u>presynaptic</u> release mechanisms has been observed to develop during chronic clorgyline treatment. This likely adaptation to increased norepinephrine levels following sustained MAO inhibitor treatment is at least in part responsible for post-synaptic adaptive changes we have observed such as β-adrenergic receptor down-regulation. Some evidence that these biochemical changes are likely to be of physiological and pharmacological importance for our understanding of the side effects and efficacy of antidepressants comes from recently-collected data indicating that self-stimulation behavior in rodents appears to be correlated with these changes. </p>		

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Chief	CN NIMH
C. S. Aulakh	Visiting Fellow	CN NIMH
J. W. Daly	Chief	LBC NIADDK
D. Pickar	Section Chief	NSB NIMH
R. P. Ebstein	Visiting Fellow	LBC NIADDK
J. F. Tallman	Section Chief	NSB NIMH
I. C. Campbell	Lecturer	Maudsley Hospital, London
S. N. Pradhan	Professor	Howard University

Project Description:

Objectives: Various antidepressant drugs enhance catecholamine functional activity rapidly in animal models; however, antidepressant effects and some side effects in man are not observable until 10-14 days or longer. This implies that central nervous system adaptive changes are likely to be important in the molecular mechanisms that are responsible both for the efficacy and the side effects of these drugs. We have engaged in a series of studies to define this process in rats with the expectation that these studies will further our understanding of the etiology and treatment of affective disorders.

Methods Employed:

Monoamine oxidase activity is determined with [^3H]-serotonin, [^{14}C]-phenylethylamine and [^{14}C]-tyramine as substrates, with subsequent separation of labelled products by ion exchange chromatography. Receptors from crude brain homogenates are measured by standard radioactive ligand assays. [^3H]-dihydroalprenolol, [^3H]-WB4101, and both [^3H]-clonidine and [^3H]-yohimbine are the specific ligands used for the measurement of β -, α_1 -, and α_2 -adrenoceptors respectively. Cyclic AMP formation is measured by the adenine prelabeling brain slice technique. Norepinephrine release experiments are performed using microsacs prepared from rat cortex and layered onto sephadex G 10 columns.

For the cardiovascular studies a pithed rat preparation is utilized with both vagus nerves cut at the neck. This procedure destroys the entire central nervous system but leaves intact the emerging nerve terminals. Plasma norepinephrine and epinephrine are assayed by a radioenzymatic thin layer chromatographic procedure using catechol-o-methyl transferase and [^3H]-S-adenosylmethionine.

Locomotor activity is measured with Animex activity meters.

For studies of self-stimulation behavior, bipolar stainless steel electrodes are implanted stereotaxically. Following surgery, rats are trained to press a lever in a Skinner box in order to receive reinforcement from intracranial electrical stimulation.

Major Findings:

After 21 days of treatment with 1 mg/kg/day of clorgyline, an A type selective monoamine oxidase inhibitor, but not after three days, there is an increase in norepinephrine release from rat brain microsacs in response to 43 mM KCl stimulation. Microsacs prepared from 21-day clorgyline treated animals also showed a marked decrease in the inhibition of norepinephrine release caused by the α_2 -selective agonist clonidine. These functional changes in norepinephrine release are accompanied by a 53% reduction in brainstem α_2 -receptor density as measured by [^3H] clonidine binding.

Also at 21 days, but not at three days, clorgyline causes significant escape from clonidine's normal suppressant effects on locomotion in the rat, a presumed behavioral concomitant to the above physiological and biochemical findings. As

it was possible that prior reports of lack of change in α_2 -receptor density following chronic tricyclic treatment resulted from the use of different radioactive ligands for α_2 -receptor measurements, we examined α_2 -receptor binding with a second ligand, the α_2 -antagonist [^3H]-yohimbine following chronic clorgyline treatment, but found similar reductions in density (57%). At the same time, despite confirming our previous findings of a decrease in β -receptor number as determined by [^3H]-dihydroalprenolol binding, no significant decreases in the responses of cyclic adenosine 3':5' monophosphate (cAMP) systems to norepinephrine stimulation were observed after 21 days of clorgyline treatment. Decreases in cAMP responses were found to require a longer duration (35 days) of clorgyline treatment.

These results provide direct physiological support for a change in the norepinephrine release mechanism and an effect on autoreceptors, preceding postsynaptic adaptive changes in the instance of at least one antidepressant, clorgyline. Under the leadership of Dr. Aulakh, we have begun to explore how these alterations might affect self-stimulation behavior as regulatory mechanisms of reinforcement seem to be altered by depression. Our findings suggest that similar adaptive changes occur in self-stimulation behavior, although they are somewhat different from those reflected in locomotor activity changes. Following chronic treatment (21 days) with clorgyline, an actual increase in self-stimulation behavior is observed, in addition to the attenuation of suppressant effect of clonidine on self-stimulation behavior.

Significance to Biomedical Research and the Program of the Institute:

We had previously proposed that an overly stringent negative feedback system could impair the capacity of the catecholamine pathways to convey information adequately during depression. The present data, particularly those pertaining to reinforcement mechanisms, support the role of some antidepressants in potentially resetting this mechanism as an important component in the molecular processes involved in antidepressant efficacy. Human studies prompted by these types of observations have already revealed changes in some functional measures of α_2 -adrenergic sensitivity following antidepressant treatment in depressed subjects. These studies of adaptive changes are also likely to have relevance for the understanding of the side effects of monoamine oxidase inhibiting antidepressants. As in the instance of antidepressant efficacy, sleep changes, behavioral side effects such as hypomania and mania, as well as cardiovascular side effects are late developing concomitants of chronic clorgyline administration in man.

Proposed Course:

During the next year, we will be attempting to extend our study of antidepressant effects on self-stimulation behavior to examine additional brain regions to evaluate the generality of our findings. We will want to look at the adaptation to the withdrawal of antidepressants, as behavioral effects are frequently seen in patients following drug discontinuation. We will want to examine some MAO-inhibitor-induced serotonin changes as well as the inter-relationship between the serotonergic and noradrenergic systems in the adaptational processes accompanying antidepressant administration.

Publications:

Cohen, R.M., Campbell, I.C., Yamaguchi, I., Pickar, D., Kopin, I.J., and Murphy, D.L.: Cardiovascular changes in response to selective monoamine oxidase inhibition in the rat. Eur. J. Pharmacol. 80: 155-160, 1982.

Cohen, R.M., Aulakh, C.S., Campbell, I.C., and Murphy, D.L.: Functional subsensitivity of α_2 -adrenoceptors accompanies reductions in yohimbine binding after clorgyline treatment. Eur. J. Pharmacol. 81: 145-148, 1982.

Cohen, R.M., Ebstein, R.P., Daly, J.W., and Murphy, D.L.: Chronic effects of a monoamine oxidase-inhibiting antidepressant: Decreases in functional α -adrenergic autoreceptors precede the decrease in norepinephrine-stimulated cyclic adenosine 3':5'-monophosphate systems in rat brain. J. Neurosci. 2: 1588-1595, 1982.

Cohen, R.M., and Campbell, I.C.: Receptor adaptation in animal models: A state change approach to psychiatric illness. In Post, R.M., and Ballenger, J.C. (Eds.): Neurobiology of the Mood Disorders, Baltimore, Williams and Wilkins Co., in press.

Aulakh, C.S., Cohen, R.M., Pradhan, S.N., and Murphy, D.L.: Self-stimulation responses are altered following long-term but not short-term treatment with clorgyline. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00335-05 CN
PERIOD COVERED <div style="text-align: center; margin-top: 10px;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Smooth pursuit eye tracking impairment and its relation to psychopathology and CNS disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Larry J. Siever Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) CN, BPB, NIMH; Harvard University; Children's Hospital, Los Angeles; Univ. of Maryland		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; margin-top: 20px;"> <p>This project was designed to evaluate psychological and psychobiological attributes associated with alterations in <u>smooth pursuit eye movements</u>. By using the <u>high-risk strategy</u> of screening a large group of <u>normal volunteers</u>, individuals with various alterations in smooth pursuit patterns were identified. Individuals with impaired tracking were compared with those with more accurate tracking using a variety of psychological and biological tests to explore the relationship of these patterns to possible psychopathology, as well as other psychological and biochemical variables. Our results indicated that <u>schizotypal psychopathology</u> marked by social isolation, suspiciousness, constricted affect, and poor rapport was significantly more likely to be present in low accuracy trackers than high accuracy trackers, findings analogous to reports of impaired tracking in the "well" relatives of schizophrenic patients. These findings were reviewed in detail in last year's report. This project was terminated in 1982, following the departure of the principal investigator, Dr. Siever, who is continuing a portion of the project at the Mt. Sinai School of Medicine and the VA Medical Center in New York.</p> </div>		

Other collaborative professional personnel engaged on the project:

D.L. Murphy	Chief	CN NIMH
T.R. Insel	Staff Physician	CN NIMH
J.R. Nurnberger	Staff Physician	BPB NIMH
J. A. Hamilton	Staff Physician	CN NIMH
P. Holzman	Assoc. Professor	Harvard University
L. Brody	Resident	Children's Hospital, L.A.
R. Coursey	Professor	Univ. of Maryland

Publications:

Siever, LM., Haier, R.J., Coursey, R.D., Sostek, A.J., Murphy, D.L., Holzman, P.S., and Buchsbaum, M.S.: Smooth pursuit eye tracking impairment: Relationship to other 'markers' of schizophrenia and psychologic correlates. Arch. Gen. Psychiatry 39: 1001-1005, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00336-04 CN
PERIOD COVERED <div style="text-align: center;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The phenomenology and treatment of obsessive-compulsive disorder in adults		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas R. Insel Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) CN, BP, CP, LPP, NIMH: Univ. of Maryland		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">3.2</div>	PROFESSIONAL: <div style="text-align: center;">1.8</div>	OTHER: <div style="text-align: center;">1.4</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Obsessive-compulsive disorder is an uncommon syndrome about which little is known. The purpose of this project is to collect basic data about the phenomenology, the psychobiology, and the treatment of this disorder. Results with several biologic variables which have previously been described as abnormal in patients with depression suggest that obsessive-compulsive disorder patients share some of the psychobiologic features of affective illness. These variables include <u>dexamethasone suppression</u>, <u>sleep physiology</u>, and <u>neuroendocrine response to clonidine</u>. Furthermore, the tricyclic antidepressant, <u>clomipramine</u>, has modest but consistent beneficial effects in obsessive-compulsive patients with or without secondary <u>depression</u>.</p>		

Other collaborative professional personnel engaged on the project:

R. M. Cohen	Staff Physician	CN	NIMH
D. L. Murphy	Branch Chief	CN	NIMH
C. F. Hoover	Social Worker	CN	NIMH
J. L. Rapoport	Unit Chief	BP	NIMH
M. Linnoila	Staff Physician	CP	NIMH
T. Zahn	Staff Psychologist	LPP	NIMH
R. D. Coursey	Professor	Dept. of	Psychology
		Univ. of	Maryland

Project Description:

Objectives: Over the past three years we have developed a comprehensive research program to investigate both psychological and biological aspects of obsessive-compulsive disorder. Our initial objectives focused on descriptive or diagnostic aspects of the syndrome: Is the disorder homogeneous or are there a variety of syndromes within this disorder with heterogeneous responses to treatment? In the second year of research our investigations focused more on the treatment of the disorder: Do patients with this disorder respond to antidepressants and if so, what biologic markers might predict drug response? In the past year, we have extended our initial treatment response findings to look more closely at the pharmacologic selectivity of this syndrome. Specifically, our earlier results with clomipramine suggested that a tricyclic antidepressant with potent inhibitory effects on the neuronal re-uptake of serotonin would reduce obsessional symptoms. In the past year we have begun to study serotonergic function in obsessional patients. One specific objective was to determine if an antidepressant drug that selectively blocks neuronal re-uptake of serotonin is more effective clinically than an antidepressant that selectively blocks re-uptake of norepinephrine.

Methods Employed:

During the first two years, obsessive-compulsive subjects were studied in both inpatient and outpatient settings. Patients who applied for the protocol were carefully screened with an extensive psychological and medical battery. Those accepted had obsessive or compulsive symptoms for at least one year independent of another psychiatric diagnosis. An initial evaluation included cognitive, projective, psycholinguistic and psychophysiological (EEG, GSR, AER) testing. Biologic measures which have proved useful in studies of affective or schizophrenic disorders were focused on here: sleep physiology, average evoked response, urinary and cerebrospinal fluid monoamine metabolites, a variety of platelet and RBC enzymes, and the dexamethasone test were all studied. Cerebral CT scans were done to investigate the possibility of gross neuropathology in this disorder. A major aspect of the study was the comparative double-blind drug trials. This trial followed a randomized crossover design with assessment by both self and observer ratings on a weekly basis. Specific biological and psychological measures were repeated during the various stages of the drug trial for correlation with clinical state.

An additional study completed during the second year examined behavioral and neuroendocrine responses to amphetamine as predictors of clinical response to clomipramine or clorgyline. The theory, borrowed from analogous studies in depressed patients, had been that those patients developing activation or euphoriant response to a single dose of d-amphetamine would be more likely to respond therapeutically to one of the drugs in the double-blind crossover study.

In the past year, the study has shifted entirely to the outpatient clinic. Local patients with obsessive-compulsive disorder are accepted if they have been ill for at least one year and are willing to stop all psychotropic medications. In place of the extensive "baseline" testing completed in previous years, patients are studied on parameters directly relevant to neurotransmitter

function. These studies include: [^3H]-imipramine binding and serotonin uptake in platelets and sampling of lumbar cerebrospinal fluid for brain amines and their metabolites. Platelet and cerebrospinal fluid studies are repeated after five weeks of treatment on one of two selective antidepressants. Each patient is randomly assigned to double-blind treatment with either zimelidine (a selective serotonin re-uptake inhibitor) or desipramine (a selective norepinephrine re-uptake inhibitor). Following a five-week trial on one of these drugs, patients cross over to a five-week trial with clomipramine.

Major Findings:

Thirty-five patients with obsessive-compulsive disorder have been studied over the past three years. A first generation of studies ended one year ago. We are currently midway through our parallel comparison of zimelidine and desipramine.

The major finding to date has been the effectiveness of clomipramine for patients with this disorder. Twelve patients studied in the double-blind cross-over comparison of clomipramine and clorgyline revealed consistent improvement on measures of obsessions, depression, and anxiety after four and six weeks of clomipramine treatment but no significant changes after equal periods of treatment with clorgyline ($n = 11$) or following four weeks of placebo administration ($n = 13$). Improvement with clomipramine treatment was not limited to patients with secondary depression, nor was it predicted by the type of obsessions (e.g., washing vs. checking). Follow-up of patients continued on clomipramine after six months and one year of treatment shows persistent and progressive improvement.

A series of biologic markers for depression have been found in a high proportion of our obsessional patients. The dexamethasone suppression test was abnormal in 7 of 26 patients (27%). Sleep EEG's in 14 obsessionals showed reduced delta (slow wave) sleep and shortened mean rapid eye movement (REM) latency, both of which have been previously associated with depression. Administration of clonidine ($n = 9$) was followed by a "blunted" plasma growth hormone rise--also previously reported in affective illness. For each of these markers, abnormalities were evident in obsessionals without secondary depression.

What predicts response to clomipramine in obsessive-compulsive disorder patients? There was not a predictive relationship between any of the biologic marker abnormalities and clomipramine response. In addition, behavioral responses to d-amphetamine administration which have been used to predict antidepressant response in affective illness, did not indicate which obsessional patients would improve on clomipramine. A chance finding from the d-amphetamine challenge study ($n = 12$) was a profound though transient improvement in obsessional symptoms following stimulant administration.

A more detailed analysis of the response to clomipramine and clorgyline has been possible with the serial psychologic and psychophysiologic tests completed across the stages of the crossover study. For instance, serial studies of galvanic skin response reveal that both clomipramine and clorgyline administration are associated with lower levels of arousal but only clomipramine decreases the

time for habituation. This change in habituation time is significantly correlated with improvement in obsessional symptoms and thus, may reflect an important aspect of clomipramine's therapeutic effect in obsessive-compulsive disorder.

Preliminary results from our more recent studies suggest that obsessional patients do not differ from normals in their platelet [^3H]-imipramine binding or uptake of serotonin. In addition, it appears that desipramine has not been effective as an anti-obsessional agent ($n = 6$), in spite of its well recognized antidepressant effects in affectively ill patients. Of five patients who have received zimelidine, only two have shown a significant improvement. These preliminary results do not support the hypothesis that serotonin uptake is abnormal in obsessional disorder or that the pharmacologic blockade of serotonin uptake is sufficient for an anti-obsessional effect. Unlike affective illness, it appears that only certain tricyclic drugs have therapeutic effects.

Significance to Biomedical Research and the Program of the Institute:

These studies of obsessive-compulsive disorder represent the most comprehensive investigations yet done in this country on this uncommon illness. The biological evidence added to clinical data points to the similarities between this syndrome and disorders of affect. That these patients respond to a tricyclic antidepressant further supports this link between the two disorders and raises new optimism for an illness which has heretofore been considered refractory to treatment.

Proposed Course:

The mechanism of clomipramine's anti-obsessional effect continues to be a major focus of interest. The relative importance of the blockade of serotonin and norepinephrine re-uptake will be further studied using zimelidine and desipramine as selective pharmacologic tools. In addition, other strategies to study serotonergic function in these patients will be pursued. In addition to extending our platelet and cerebrospinal fluid studies, we hope to soon begin selective pharmacologic challenges to the serotonergic system to assess post-synaptic serotonin receptor sensitivity. By assessing the receptors, the platelet uptake, and the turnover (CSF metabolites) of serotonin, we may be able to determine if there is some dysfunction of this neurotransmitter system in obsessive-compulsive disorder and if such a dysfunction exists, how it may explain the beneficial effects of clomipramine.

Publications:

Insel, T.R., Gillin, J.C., Moore, A., Mendelson, W., Loewenstein, R.J., and Murphy, D.L.: The sleep of obsessive-compulsive disorder patients. Arch. Gen. Psychiatry 39: 1372-1377, 1982.

Insel, T.R., Hamilton, J., Guttmacher, L., and Murphy, D.L.: D-amphetamine in obsessive-compulsive disorder. Psychopharmacology, in press.

Insel, T.R., Donnelly, E.F., Lalakea, M.L., Alterman, I.S., and Murphy, D.L.: Neurological and neuropsychological studies of patients with obsessive-compulsive disorder. Biol. Psychiatry, in press.

Insel, T.R., Hoover, C., and Murphy, D.L.: Parents of patients with obsessive compulsive disorder. Psychol. Med., in press.

Siever, L., Insel, T.R., Jimerson, D., Lake, C.R., Uhde, T.W., Aloï, J., and Murphy, D.L.: Blunted growth hormone response to clonidine in obsessive-compulsive patients. Br. J. Psychiatry 142: 184-187, 1983.

Insel, T.R., Murphy, D.L., Cohen, R.M., Alterman, I., Linnoila, M., and Kilts, C.: Obsessive-compulsive disorder: A double-blind treatment trial of clomipramine and clorgyline. Arch. Gen. Psychiatry, 40: 605-612, 1983.

Insel, T.R., Alterman, I., and Murphy, D.L.: Anti-obsessional and antidepressant effects of clomipramine in the treatment of obsessive-compulsive disorder. Psychopharmacol. Bull. 18: 115-117, 1982.

Insel, T.R., Roy, B., Cohen, R.M., and Murphy, D.L.: Possible development of the serotonin syndrome in man. Am. J. Psychiatry 139: 954-955, 1982.

Linnoila, M., Insel, T.R., Kilts, C., Potter, W.Z., and Murphy, D.L.: Plasma steady-state concentrations of hydroxylated metabolites of clomipramine. Clin. Pharmacol. Ther. 32: 208-211, 1982.

Insel, T.R., and Pickar, D.: Naloxone exacerbates obsessive doubt. Am. J. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00337-04 CN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Neuropharmacology of neuroendocrine and neurotransmitter regulatory mechanisms</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Thomas R. Insel Staff Physician, Clinical Neuropharmacology Branch, NIMH</u>		
COOPERATING UNITS (if any) <u>Wisconsin Primate Lab, Madison; Centre for Reprod. Biology, Edinburgh; Univ. of California, San Diego; CN, LCS, CP, LPP, NB, NIMH; DBEB, NICHD</u>		
LAB/BRANCH <u>Clinical Neuropharmacology Branch</u>		
SECTION 		
INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>2.3</u>	PROFESSIONAL: <u>1.5</u>	OTHER: <u>0.8</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We have continued our studies of <u>cerebrospinal fluid (CSF)</u> levels of various hormones, peptides, monoamines and monoamine metabolites in rhesus monkeys and man. An investigation of CSF <u>serotonin</u> and <u>melatonin</u> concentrations sampled over 24-hour periods revealed a 20-fold diurnal variation in serotonin in rhesus monkeys. Serotonin concentrations peaked at midnight, and followed a similar time pattern as that for CSF melatonin. Both rhythms were essentially obliterated by continuous light and by the β-adrenergic antagonist, propranolol, indicating similar regulatory mechanisms for both substances. Studies with prolactin have demonstrated a blood-CSF gradient for this hormone. In addition, a ventricular-lumbar gradient within the CSF is not present under baseline conditions but develops following prolactin release elicited by treatment with thyrotropin stimulating hormone. Prolactin release elicited by the cholinergic agent, <u>physostigmine</u>, may be modulated by hypothalamic <u>β-endorphin</u> release. Studies with the new <u>benzodiazepine</u> receptor antagonist, <u>β-carboline ethyl ester</u> in rhesus monkeys have suggested a new pharmacologic model of "<u>anxiety</u>" and provided an opportunity to study the neuroendocrine and catecholamine changes that accompany clinically-relevant behavior in primates. </p>		

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Branch Chief	CN NIMH
R. M. Cohen	Staff Physician	CN NIMH
L. Tamarkin	Staff Fellow	DBEB NICHD
S. P. Markey	Unit Chief	LCS NIMH
M. Linnoila	Staff Physician	CP NIMH
D. Jimerson	Staff Physician	LCS NIMH
T. Zahn	Staff Psychologist	LPP NIMH
S. Paul	Branch Chief	NB NIMH
N. Kalin	Staff Physician	Wisconsin Primate Lab, Madison, WI
P. Taylor		Centre for Reprod. Biology, Edinburgh
S. C. Risch	Staff Physician	Univ. of California San Diego

Project Description:

Objectives: The discovery that a multitude of peripheral peptide hormones are present in high concentrations in the brain has led to an entire field of inquiry into the modulatory interactions between peptides, hormones and the classical monoamine neurotransmitters. This project has focused on the measurement of peptide hormones and monoamines and their metabolites in cerebrospinal fluid in an attempt to evaluate (a) diurnal changes; (b) define the relationship between plasma and CSF peptide levels; (c) evaluate their concentrations at different levels of CSF (i.e., lateral ventricular versus lumbar); and (d), in particular, to assess the effects of drugs which are known to affect monoamines using biochemical and, more recently, behavioral measurements.

Methods Employed:

Cerebrospinal fluid from non-human primates is collected by means of indwelling lumbar or lateral ventricular cannulae for continuous flow into a refrigerated fraction collector. Monkey plasma is obtained either by use of indwelling venous catheters or by femoral venipuncture following ketamine induced anesthesia. The following hormones are measured by radioimmunoassay: cortisol, prolactin, growth hormone, β -endorphin and melatonin (in collaboration with Larry Tamarkin, NICHD). Serotonin is measured by capillary mass spectrometry.

In the studies evaluating a non-human primate model for anxiety, β -carboline ethyl ester (β -CCE) was administered intravenously according to a randomized dosage schedule (0-500 μ g/kg). Behavior was rated in a double-blind manner using a scale developed from earlier β -CCE studies as well as from previously published studies of "anxiety-like" behavior in rhesus monkeys. Blood pressure and heart rate were monitored continuously using an ankle cuff. Plasma was obtained from an indwelling venous catheter for measurement of cortisol (by radioimmunoassay) and MHPG (by mass spectrometry, in collaboration with David Jimerson, LCS). Following a dose-response study, the lowest dose necessary for behavioral and physiologic activation was administered in a subsequent study to assess the effects of pretreatment with each of three pharmacologically different anxiolytics (diazepam 0.5 mg/kg, clonidine 10 μ g/kg and propranolol 3 mg/kg).

Major Findings:

Studies of the diurnal variation in CSF serotonin in rhesus monkeys have been extended by Nancy Garrick and Larry Tamarkin to include a larger number of animals and to evaluate regulatory factors in serotonin release. These studies have revealed that concentrations of serotonin in CSF reach nocturnal peaks that average 20 times the mean daytime values and range up to 70 times higher. The release of serotonin into CSF is temporally similar to that of melatonin but the concentration of serotonin at night exceeds that of melatonin 50-fold. To determine whether the diurnal rhythm for CSF serotonin is regulated like the diurnal rhythm for melatonin, we studied rhesus monkeys during normal light-dark cycles and under conditions that alter the release of pineal melatonin (e.g. constant light and following the administration of the β -adrenergic antagonist propranolol). The large nocturnal elevations in CSF serotonin and melatonin were abolished by exposure to constant light. Returning the animals to a light-dark cycle immediately reestablished the nocturnal rise in serotonin and melatonin.

Propranolol also markedly reduced the serotonin and melatonin elevations in monkeys kept on a light-dark schedule but given the drug one hour before darkness. The similar suppressive effects of light and propranolol on nocturnal elevations of serotonin and melatonin indicate similar regulatory influences on these two indoleamines.

A significant diurnal variation in CSF prolactin, which is similar to the rhythm in plasma, has been demonstrated in the rhesus monkey. Since CSF prolactin undergoes regular variations in concentration which are thought to have physiological importance, the relationships between blood and CSF concentrations of prolactin were studied in repeated simultaneous blood and CSF samples by Ned Kalin and coworkers. Following administration of thyrotropin releasing hormone there was an increase in both plasma and lumbar CSF prolactin concentrations. The increase in CSF prolactin concentrations were delayed until 60 minutes after peak plasma concentrations were attained. Prolactin concentrations were compared in simultaneous lateral ventricular and lumbar CSF samples. No difference was found under baseline conditions; however, a ventricular-lumbar prolactin concentration gradient became apparent after stimulation by thyrotropin releasing hormone. These studies demonstrate that changes in plasma prolactin concentrations are reflected in CSF concentrations, and they suggest that a significant blood-CSF barrier exists for prolactin which may enter the CSF selectively via the ventricles.

Studies of neuroendocrine function in humans have demonstrated a highly significant increase in plasma β -endorphin and prolactin immunoreactivity following physostigmine infusion, but not after placebo. Changes in plasma concentrations of β -endorphin and prolactin subsequent to physostigmine were highly correlated. Significant increases in plasma β -endorphin and prolactin also occurred after arecoline, but not after placebo, with changes in plasma β -endorphin and prolactin again being highly correlated. These high correlations between increases in plasma β -endorphin and prolactin in man might provide a useful model for understanding the effects of centrally active cholinergic agents on anterior pituitary prolactin regulation, in addition to being an interesting example of peptidergic modulation of primary neurochemical mechanisms involved in hypothalamic-pituitary prolactin regulation.

β -Carboline-3-carboxylic acid ethyl ester (β -CCE) is a high affinity benzodiazepine receptor ligand with potent behavioral and physiologic effects in primates. Dose-related increases in behavioral agitation, plasma cortisol, blood pressure, and heart rate were observed after administration of doses between 50 and 500 μ g/kg of β -CCE to rhesus monkeys. All of these effects were blocked by pretreatment with diazepam. Pretreatment with clonidine and propranolol, both of which have been reported to have anxiolytic actions, attenuated only selective aspects of the response to β -CCE.

Significance to Biomedical Research and the Program of the Institute:

Serotonin released in large quantities at night may act as a cerebro-ventricular hormone to influence brain and pituitary function, and may be an important measure of physiological and behavioral states relevant to psychopharmacology. The behavioral, endocrine and physiological effects of low doses

of β -CCE in monkeys are similar to those observed in anxious patients or normals under anxiety-provoking or stressful situations, and thus administration of benzodiazepine receptor antagonists such as β -CCE may provide a valid and reproducible primate model of human anxiety which could be used to investigate specific biological aspects of anxiety.

Proposed Course:

Since nocturnal, light-regulated flooding of serotonin through the cerebro-ventricular system may play a role in other diurnal physiological alterations, we are currently studying sleep-wake parameters and neuroendocrine rhythms in monkeys.

Publications:

Kalin, N.H., Burns, S., Risch, S.C., Cosgrove, S.A., Warden, D.A., and Murphy, D.L.: The relationship between blood and cerebrospinal fluid prolactin in non-human primates. Life Sci. 31: 159-163, 1982.

Risch, S.C., Janowsky, D.S., Siever, L.J., Judd, L.L., Rausch, J.L., Huey, L.Y., Beckman, K., Cohen, R.M., and Murphy, D.L.: Cholinergically stimulated hypothalamic β -endorphin modulation of anterior pituitary prolactin release in humans. Peptides 3: 319-322, 1982.

Insel, T.R., and Goodwin, F.K.: The promises and problems of diagnostic tests in psychiatry: The dexamethasone suppression test as a case example. Hosp. Commun. Psychiatry, in press.

Insel, T.R., and Goodwin, F.K.: The dexamethasone suppression test as a predictor of relapse. In Hirschfeld, R. (Ed.): The Clinical Utility of the Dexamethasone Suppression Test, in press.

Taylor, P.S., Garrick, N.A., Burns, R.S., Tamarkin, L., Murphy, D.L., and Markey, S.P.: Diurnal rhythms of serotonin in monkey cerebrospinal fluid. Life Sci. 31: 1993-1999, 1982.

Garrick, N.A., Tamarkin, L., Taylor, P.L., Markey, S.P., and Murphy, D.L.: Light and propranolol suppress the nocturnal elevation of serotonin in the cerebrospinal fluid of rhesus monkeys. Science, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00338-03 CN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Families of origin in obsessive-compulsive illness		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Carol F. Hoover Social Worker, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) CN, NIMH		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.3	PROFESSIONAL: 0.2	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 10px 0;">This study explores the etiological context of <u>family relations</u> in <u>obsessive-compulsive disorder</u>, along with the <u>incidence</u> of <u>obsessive-compulsive disorder among relatives</u>.</p> <p style="margin: 10px 0;">Family data have been collected on 174 relatives of ten severely obsessive-compulsive patients. These rather isolated families had cultures which emphasized cleanliness and perfection, but other family members did not develop rituals or obsessive rationales as the patient did. Typically one or both parents in an unfulfilled marriage directed symbiotic needs toward the patient. Parents and offspring became trapped in an increasingly powerless struggle against symptoms which acted as a barrier to closeness, but also prevented the patient from developing an autonomous existence.</p>		

Project Description:

Objectives: This study is intended to illuminate the following areas: (1) The incidence of obsessive-compulsive disorder in relatives of late adolescent and adult patients with severe forms of the illness. (2) The psychosocial milieu presented in the families of origin. (3) Similarities or differences in personality, attitude, and coping styles between (a) patients and (b) members of their nuclear family. (4) Patterns of family interaction, with special reference to parent/child and husband/wife relationships. (5) Observational comparisons between the immediate families of young schizophrenics and the families of severely obsessive-compulsive patients.

Methods Employed:

Family data were collected on 10 severely obsessive-compulsive patients, their 20 parents, 20 siblings, 3 offspring, and 131 second and third degree relatives. At least two relatives of each patient were interviewed, often over a period of months, and in most cases the families also participated in conjoint sessions.

Major Findings:

There were no relatives who suffered from classical obsessive-compulsive disorder, though other mental illnesses occurred in 11.6% of first-degree relatives. The families were typically somewhat isolated from their communities and had cultures of super-cleanliness, over-meticulousness and the like, which had persisted through the generations. Although this psychosocial milieu enhanced the development of obsessive-compulsive symptoms in an offspring, neither parents nor siblings adopted the patient's irrational explanations of concerns, nor did their standards of performance and cleanliness overwhelm them and become transformed into rituals. The patient's symptoms generally puzzled and frightened their relatives, although parents were often bullied into indulging an offspring's bizarre practices.

Along with an isolation from exterior contacts, there were severe conflicts within the nuclear family regarding intimacy and closeness. Relationships between the parents were unfulfilled, disappointing, strained, distant, or furiously argumentative. One or both parents concentrated more upon an offspring than upon each other, seeking an intense symbiotic involvement which was thwarted by the distancing obsessive-compulsive symptomatology in a son or daughter. The offspring simultaneously "used" the parental desires to achieve increased family power through tantrums or other pressure tactics, yielding a parental helplessness and demoralization which contributed further to the developing illness. This engulfing symptomatology in turn made the patient helplessly dependent upon parental services, and unable to live apart from a caretaking family.

The question may be raised whether some heritable factor might contribute to the obsessional "culture" of over-meticulous habits observed in successive generations in these families, combining perhaps with elements in family relationships to produce obsessive-compulsive disorder in a vulnerable offspring.

Significance to Biomedical Research and the Program of the Institute:

This research attempts to develop a comprehensive pattern for evaluating familial factors in a severe and puzzling disorder. These elements of family relations and incidence are essential to an understanding of obsessive-compulsive disorder.

Proposed Course:

Analysis is continuing, and material is being prepared for submission to a professional journal. Meanwhile, a related study, "Parents of Patients with Obsessive Compulsive Disorder" (referenced in Z01 MH 00336-04 CN), which includes data obtained through administration of the Leyton Obsessionnal Inventory to patients and parents, has been accepted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00339-02 CN
PERIOD COVERED <div style="text-align: center; margin-top: 10px;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The neuropharmacology of cognition		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Robert M. Cohen Staff Physician, Clinical Neuropharmacology Branch, NIMH		
COOPERATING UNITS (if any) CN, LPP, NSB, NIMH		
LAB/BRANCH Clinical Neuropharmacology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">0.6</div>	PROFESSIONAL: <div style="text-align: center;">0.3</div>	OTHER: <div style="text-align: center;">0.3</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Memory problems are a common complaint in the affective disorders. In work recently completed with Dr. Weingartner of the Laboratory of Psychology and Psychopathology, we were able to demonstrate close relationships among increasing severity of <u>depression</u> and decrements in both motor and memory tasks in affectively disordered patients. The results suggested that a deficit in the central <u>motivational state</u> of depressed subjects might account for their performance problems. To expand these findings of a relationship between central motivational state and cognitive performance, we chose to look at the opiate system in man, as <u>endogenous opiates</u> seem to be important in the regulation of reinforcement and cognition in animals, and of mood in man. To appropriately assess the system a high dose naloxone strategy was required. At high doses (> 2 mg/kg), naloxone increased depression and anxiety ratings in normals and produced a decrease in memory performance. The pattern of memory changes, however, was somewhat different from those predicted from our work with depressed patients.</p>		

Other collaborative professional personnel engaged on the project:

D. L. Murphy	Branch Chief	CN	NIMH
H. Weingartner	Unit Chief	LPP	NIMH
D. Pickar	Section Chief	NSB	NIMH
T. R. Insel	Staff Physician	CN	NIMH

Project Description:

Objectives: Memory deficits have been associated with a number of neurotransmitter pathways in specific disease states and in drug-induced states in normals. Although memory performance is a somewhat specialized form of behavior, the study of memory performance requires an understanding of the interaction of motivation, drive and attention with what may be the specific physiological events of remembering (the engram). Therefore, the study of cognition in depression and other neuropsychiatric illnesses may help to elucidate the specific mechanisms whereby the physiologic and biochemical substrates of these illnesses influence the central motivational state and its interaction with the specific processes involved in behavior in general, and in particular of memory. It is probably not coincidental that primary neuropsychiatric illnesses (SLE, Alzheimer's and Korsakoff's) are associated with mood disorders. Therefore, understanding the interrelationships between mood and cognition should aid in the treatment of some of the deficits in these disorders. The use of drugs to manipulate neurotransmitter systems in both patients and normals is viewed as a useful strategy in understanding these relationships.

Methods Employed:

Behavioral and Psychological Assessment: Diagnoses of major affective illness in each of the patients is made on the basis of Research Diagnostic Criteria with the aid of the Schedule for Affective Disorders and Schizophrenia. Degree of depression is measured by the nursing staff utilizing the Bunney-Hamburg 15-point ward rating scale, by physicians with the Hamilton rating scale, and by the subjects using the Beck Depression Inventory and the Profile of Mood States.

The diagnosis of dementia is based in part on the Hugh's Scale from Washington University which incorporates both Pfeiffer's short portable mental status questionnaire and the Blessed Scale in addition to other objective information obtained from an informant.

A number of tests of memory have been employed. These include measurements of short-term memory of items consisting of three consonants, e.g., MXP, working memory, recognition memory, and free recall memory predominantly. These tests are primarily verbal in nature, but in some instances visual memory is tested. Also a motor task measurement of sustained effort is employed wherein a subject's peak and subsequent sustained effort in squeezing a dynamometer is measured.

Biological Assessment: Plasma, platelets, urine and cerebrospinal fluid are collected for measurement of enzymes, levels of biogenic amines and their metabolites. The dexamethasone suppression test and the TRH stimulation tests are also used.

Major Findings:

As reported last year, memory performance in depressed subjects was strongly associated with decrements in motor performance and with severity of depression. Greatest depression-related impairment was found in those cognitive and motor tasks that required sustained effort.

In vivo experimental effects of opiate agonists and antagonists suggest that the endogenous opioid system is a modulator of the acquisition and retention of environmental events in animals. In man the opiate agonists appear to be important in the modulation of attention, pain and pleasure states. Although hormonal and nociceptive changes had been reported in man, no consistent alterations in cognition and mood had been observed following administration of the opiate antagonist naloxone. In conjunction with Drs. Martin Cohen and David Pickar of the Clinical Neuroscience Branch and Dr. Weingartner of the Laboratory of Psychology and Psychopathology, we undertook a study of the effects of high doses of naloxone on human behavior and physiology. Increasing intravenous doses of naloxone (0.3 mg/kg, 1 mg/kg and 2 mg/kg) were administered to normal subjects. Naloxone at 2 mg/kg, but not at lower doses, impaired aspects of memory as measured by a verbal learning task which assessed the direct free recall ($p < 0.05$) and recognition ($p < 0.01$) of presented vs. nonpresented words of a single category (effortful processing) and the monitoring of the frequency of such presentations (automatic processing) ($p < 0.01$). At the same time "working" memory was left unaffected. In conjunction, with other data collected in these studies, physiological changes (blood pressure, temperature, respiratory and hormonal alterations) and behavioral changes (increasing anxiety, irritability and depression) in the absence of fatigue suggests that the opioid system plays a role in normal physiology, mood states and memory processes in man.

Significance to Biomedical Research and the Program of the Institute:

Cognitive changes frequently accompany the mood disturbances that characterize the affective disorders, with laboratory observations of depressed patients showing a positive correlation between the degree of impairment and the intensity of depression. So striking are these changes that they are frequently assigned a central role both in the etiology and treatment of depression. The finding of a close relationship between the decrements in performance in both motor and cognitive tasks in depression leads one to propose the parsimonious explanation of a single deficit in the central motivational state in depression. In addition, the methods developed can be usefully employed in studying other cognitively impaired populations to dissect the issues of motivation and reinforcement from other types of impairments (e.g., in Alzheimer's and Korsakoff's patients). Drug effects on cognition in these patients must be critically analyzed since motivation and effort may underlie cognitive changes which would otherwise appear to be direct effects on the memory processes themselves.

Proposed Course:

We have been successful in gathering a population of subjects with a variety of neuropsychiatric disorders for a continuing in depth study of mood and the effects of neuropharmacologic challenge strategies on mood and cognition. As naloxone has recently been reported to alter cognition favorably in demented subjects, it is a natural choice given the background of our former studies with naloxone to pursue this in our study population. In addition drugs that are specifically known to alter mood, e.g. antidepressants, are another logical choice with which to study the relationship of mood to cognition. We have begun

the study of the selective MAO B-inhibiting antidepressant deprenyl in this regard. Effects on cognition and the pattern of cognitive abnormalities observed in those subjects will be correlated with neurochemical findings.

Publications:

Cohen, R.M., Weigartner, H., Smallberg, S.A., Pickar, D., and Murphy, D.L.: Effort and cognition in depression. Arch. Gen. Psychiatry 39: 593-597, 1982.

Cohen, R.M., Cohen, M.R., Weingartner, H., Pickar, D., and Murphy, D.L.: High-dose naloxone affects task performance in normal subjects. Psychiatry Res. 8: 127-136, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00446-14 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Inpatient clinical studies of affective illness		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas A. Wehr, M.D., Acting Chief, CP, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.0	PROFESSIONAL: 2.5	OTHER: 2.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The overall objective of this integrated group of research studies is a more comprehensive understanding of the pathophysiology of <u>unipolar and bipolar affective disorder</u>, as well as the more recently described <u>seasonal affective disorder</u>. The further development of our outpatient department has enabled us to greatly expand the investigative capacities of the inpatient unit. In contrast with previous years, the average admission for a patient with depression is short, approximately three weeks. During this time patients undergo a series of <u>neuroendocrine, neurochemical and sleep and circadian</u> studies which will enable us to further characterize their depressions. Patients with major affective disorder undergo a trial of <u>partial sleep deprivation</u> which may produce immediate therapeutic effects. Patients who are not improved are then started on drug therapy as part of double-blind investigations which are continued in the outpatient department. Shorter hospital stays have enabled us to increase the number of patients studied and to expedite therapy. Our ability to identify diagnostic subgroups, predictors of response and to test specific hypotheses have all been exhausted. </p> <p> At the same time we have expanded our <u>longitudinal studies of affective patients</u> in two ways: (1) descriptive studies of <u>rapid cycling manic depressives in entrained conditions</u>; (2) studies of <u>selected patients in conditions where they are isolated from time cues (temporal isolation)</u>. </p> <p> The primary treatment modalities under investigation are: (1) <u>environmental manipulations</u> such as <u>partial sleep deprivation and phototherapy</u>; (2) <u>tricyclic antidepressants</u> showing specificity for given neurotransmitter systems; and (3) trials of <u>euthyroid and hypermetabolic doses of thyroxine</u> in <u>rapid cycling manic depressives</u>. </p>		
(193)		

Other Professional Personnel:

David Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Norman Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
William Potter, M.D., Ph.D.	Chief, Unit on Clinical Psychopharmacology	CP/NIMH
Matthew Rudorfer, M.D.	Clinical Associate	CP/NIMH
Barbara Parry, M.D.	Clinical Associate	CP/NIMH
Steven James, M.D.	Clinical Associate	CP/NIMH
Judith Kline	Clinical Social Worker	CP/NIMH

Project Description:

This description attempts to provide a comprehensive view of the inpatient clinical studies of manic-depressive illness. In this group of studies, the overall objective is a more comprehensive understanding of the pathophysiology of unipolar and bipolar affective disorders, particularly in relation to specificity of diagnosis, existence of clinically or biologically identifiable subgroups, the nature of predisposing factors, and the interrelationship between pharmacological and psychodynamic factors in clinical improvement.

Traditionally, the focus of our specialized resources has been the longitudinal study of episodes of affective illness before, during and often following a variety of therapeutic interventions. Initially, studies focused on classical, discrete episodes of mania or endogenous depression; more recently, our inpatient resources have been devoted increasingly to individuals with rapid-cycling bipolar illness. During the past year we continued to expand the "brief inpatient evaluation" program for depression in which outpatients are admitted to the unit for two to three weeks of pretreatment studies and are then discharged to be treated as outpatients. This approach enables us to evaluate a much broader spectrum of depressive disorders, using the methodologies previously applied to the endogenous depressions, without seriously compromising our ongoing longitudinal studies of entire illness episodes. A major new group of outpatients with seasonally recurring depression has been studied during summer and winter, and before and after experimental treatment with light. Both the total number of patients studied and number of individual projects has increased. The resulting impact on the ward milieu has been well managed.

Central to an effective program of inpatient studies are regular and reliable procedures for gathering data. A variety of variables are monitored continually during a patient's hospital stay. Trained nursing staff members complete twice-daily global ratings, assessing depression, mania, psychosis, anger, and anxiety. Patients complete self-rating forms twice a day. Specialized scales, such as the Hamilton Depression Scale, the Beck Depression Inventory, or the Symptom Checklist (SCL-90), are used for particular studies. Information regarding families is obtained through ongoing contact between the social workers and the patient's relatives.

Behavioral data are continually collected and quantified. Sleep is recorded at half-hour intervals throughout the night. Motor activity is recorded continuously using solid-state wrist activity monitors (see Project Z01 MH 00450-09 CP),

allowing the application of sophisticated mathematical techniques such as spectral analysis to define hitherto unrecognizable patterns in the data. Finally, samples of plasma and urine are collected at regular intervals and stored for subsequent analysis, providing a "bank" of biochemical specimens from various mood states and treatment. We are currently developing an optical scan, computer-based system of automated record keeping for ratings, procedures, etc. It is anticipated that this system will greatly facilitate management of research protocols and data analysis, and will be considerably less labor-intensive than the existing data management systems.

In addition, specific cross-sectional studies are scheduled to provide information about the patient's pretreatment biochemical state and the biochemical changes associated with changes in mood and treatment modality. Among these cross-sectional studies are: baseline neuroendocrine values, circadian neuroendocrine patterns, and responses to neuroendocrine challenges (dexamethasone, saline, and TRH infusions); circadian patterns of activity and temperature; sleep recordings; and cerebrospinal fluid and urinary monoamines and monoamine metabolites.

Since descriptive and diagnostic dimensions are crucial to the meaningful interpretation of psychobiologic data, work on the standardization of these dimensions has continued. Use of the Research Diagnostic Criteria (RDC) (described in detail in the July 1976 - September 1977 report on Project Z01 MH 00446-08 CP) is now routine. Discharge review conferences are used to establish both a final RDC diagnosis and assess the extent to which each patient has "typical endogenous" depressive episodes. In order to facilitate this task, a subjective "typicality" scale is assessed and a record of specific endogenous features is made.

After the evaluation phase of hospitalization, a variety of investigational interventions are employed. Environmental manipulations such as sleep shifts and exposure to bright light have played an increasingly prominent role in the overall research program.

A variety of pharmacologic studies continue, and while these are initiated on the inpatient unit they are frequently completed in the outpatient department. All drug trials are conducted under double-blind conditions.

Results of these interventions are described in greater detail in the individual project reports. Recently, greater use is being made of outpatient volunteers as controls in the various studies. In practice this population enables our use of beds to be more cost-effective than is the case with the traditional live-in volunteers. A major new development has been the establishment of a special room in which patients' and normal volunteers' circadian rhythms can be studied in isolation from external time cues which normally mask and entrain the rhythms. A computer-based monitoring system is being developed for the temporal isolation facility, and several new projects involving the facility are under way.

A second major development is the incorporation of a sleep monitoring facility into the ward. Transfer of the sleep laboratory to our inpatient unit will facilitate nursing and medical oversight of subjects whose sleep is recorded

and will foster the integration of sleep physiology and circadian rhythm approaches to research in affective illness.

Significance to Biomedical Research and to the Program of the Institute:

By their very nature the projects of the Clinical Research Unit are designed to enlarge our understanding of the causes and treatment of affective disorder. These projects are distinguished by their attempts to develop new types of anti-depressant treatment modalities, such as drugs with relatively specific effects on individual neurotransmitter systems, and novel non-pharmacological approaches involving manipulations of the sleep-wake cycle and the light-dark cycle. Temporal isolation experiments may yield information about fundamental causes of some of the disturbances present in affective illness.

Proposed Course:

In the future, clinical studies on the research unit will be organized in such a way as to expand and integrate studies of sleep and biological rhythms in affective disorder. Facilities for temporal isolation studies of circadian rhythms will be upgraded.

A new project involving hypermetabolic doses of thyroid to treat rapid cycling manic-depressives is envisaged. These patients, along with a large new group of seasonal depressives, will continue to be a focus of research.

Publications:

Jamison, K.R. and Goodwin, F.K.: Psychotherapeutic treatment of manic-depressive patients in lithium. In The Interrelationship of Psychotherapy and Psychopharmacology. Greenhill, M. and Gralnick, A. (eds.): McMillan Company, New York, Chapter 5, pp. 53-79, 1982.

Lewy, A.J. and Goodwin, F.K.: Mental effects of reserpine in man: A review. In Psychiatric Complications of Medical Drugs. Shader, R.I. (ed.): Raven Press, New York, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00447-14 CP
PERIOD COVERED <u>October 1, 1982, through September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Amine neurotransmitters and metabolites in mental illness</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>William Z. Potter, M.D., Ph.D., Clinical Psychobiology Branch, NIMH</u>		
COOPERATING UNITS (if any) <u>Laboratory of Clinical Science, NIMH; Clinical Neurosciences Branch, NIMH; Biological Psychiatry Branch, NIMH</u>		
LAB/BRANCH <u>Clinical Psychobiology Branch</u>		
SECTION		
INSTITUTE AND LOCATION <u>NIMH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>2.75</u>	PROFESSIONAL: <u>1.75</u>	OTHER: <u>1.0</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Since the mid-1960's alterations of amine neurotransmitter systems (norepinephrine (NE), serotonin (5HT) and dopamine (DA)) have been indirectly implicated in the pathophysiology of the major mental illnesses, depression and schizophrenia. Although direct demonstrations of abnormalities have not been consistently possible except for the separation of unipolar and bipolar depression on the basis of the NE metabolite MHPG in urine, findings using newer techniques and strategies continue to implicate the amine neurotransmitters. We have continued to study cerebrospinal fluid (CSF), plasma and urine from drug-free patients with affective illness and schizophrenia using more sensitive and comprehensive characterization of the neurotransmitter systems. Careful control of sources of variance and selection of appropriate age- and sex-matched controls have resulted in several new lines of evidence consistent with neurotransmitter dysregulation in mental illness: </p> <ol style="list-style-type: none"> 1. Although not clearly seen in previous studies, NE and MHPG in CSF are now found to be lower in bipolar than unipolar depression. 2. Unstimulated resting plasma NE and MHPG is either no different or lower than in appropriately matched volunteers (contradicting earlier reports), whereas stimulated values are proportionally higher. 3. Urinary MHPG alone can be a misleading marker; a more robust measure of NE turnover can now be provided through simultaneous measures of other important metabolites, normetanephrine and VMA as well. 4. Indices of 5HT and DA function in the CSF are highly interdependent and related to those of NE under some conditions; schizophrenics with wide ventricles have lower concentrations of both 5HT and DA metabolites and respond poorly to treatment. Measures of the same systems in plasma are in a developmental change but preliminary results suggest that the DA metabolite, HVA, in plasma shows abnormal variance in schizophrenic patients. <p style="text-align: center;">(199)</p>		

Other Professional Personnel:

Rex W. Cowdry	Chief, Outpatient Unit	CP/NIMH
Markku Linnoila	Staff Psychiatrist	CP/NIMH
Matthew Rudorfer	Staff Fellow	CP/NIMH
Mika Scheinin	Visiting Fellow	CP/NIMH
Thomas A. Wehr	Chief, Clinical Research Unit	CP/NIMH
David Rubinow	Staff Psychiatrist	BP/NIMH
David Jimerson	Staff Psychiatrist	LCS/NIMH
David Pickar	Chief, Section on Clinical Studies	NS/NIMH

Project Description:

The characterization of the functional state of three amine neurotransmitter (NT) systems, NE, 5HT and DA, in depression and other major psychiatric illnesses such as schizophrenia is an ongoing effort in the intramural program. Over a decade of method development and clinical studies has led to the identification of numerous sources of variance which we have only recently been able to control. In many instances, it remains an open question whether appropriate controls are possible.

Simultaneous studies, particularly in depressive illness of neuroendocrine state, peptidergic systems and post-synaptic receptors complement rather than supplant studies of the neurotransmitters themselves and still tend to be conceptualized as either dependent or co-variants of NT function. Even DST escape can be viewed as an HPA abnormality reflecting regulation of the adrenergic/noradrenergic system(s).

Continued expansion of our understanding of the regulation of these NT systems in both healthy volunteers and psychiatric patients seems certain to at least provide tools to subtyping psychiatric illness and predicting response to treatment. Following this classic approach with new techniques still holds the hope of pointing to the underlying pathophysiology of at least some mental illness.

Methods:

Clinical: Selection of subjects paying particular attention to such issues as age of onset, frequency of recurrence of episodes, and family history is given great emphasis. Whenever feasible, extended (over 1 month) drug-free periods are required before biological samples are obtained--a 3-week period is our current minimum. Patients are also characterized according to length on a low monoamine diet as well as number of days in hospital. This latter parameter is of particular interest since many depressed patients are studied after brief (sometimes only overnight) hospitalization and then transferred to outpatient status.

"Control" subjects are drawn primarily from hospitalized age- and sex-matched individuals who are asked to be on diet. It appears, however, that for comparisons of urine and CSF hospitalization can be a critical variable. Therefore, a comparison of "controls" under different conditions is indicated as an essential component of our design.

Laboratory:

More sensitive, efficient and comprehensive measures of NTs and their metabolites are constantly being developed as improved HPLC pumps and detectors become available. The synthesis and application of better internal standards has been equally important. GC-MS is still required for analysis of transmitters and related compounds in urine. Specifically, we have measured a number of dietary amino acid products such as tyramine and phenyl ethylamine which might have biologic activity in the CNS.

Major Findings:

1. The use of NE and its metabolites to distinguish subtypes of depression has been extended. In our most recent groups of unipolar and bipolar patients, urinary MHPG alone showed only a trend toward reduction in the bipolars; both NE and MHPG in the CSF, however, were clearly and significantly lower in the bipolars than in the unipolars, providing additional evidence that bipolar depression is a relatively hypoadrenergic state both when compared to unipolar depression and to mania.

2. For the first time we have been able to obtain interpretable measures of noradrenergic function in the plasma of depressed patients and compare it to age- and sex-matched controls. In contradistinction to preliminary reports by other groups, we find no elevation of resting (supine) plasma NE (or MHPG) but do show an exaggerated NE response to postural change from lying to standing. These findings suggest that other forms of acute stress (e.g., circumstances of doing the study) might produce relatively higher elevations of plasma NE in depressed subjects. Since physiologic function (pulse and blood pressure) did not follow the relative excess NE increase, we interpret this as evidence of dysregulation of the NE system in depression rather than some compensatory increase.

3. Other sources of variance continue to be identified, such as the high correlation between tyramine and NE + metabolite excretion in urine. There appears to be an ordering and/or environmental effect on measures of NTs in plasma, CSF and urine, especially for non-hospitalized volunteers. New systematic studies of hospitalized vs. non-hospitalized middle-aged volunteers is necessary before apparent differences to patients can be interpreted with confidence.

4. Collaborative studies with other Branches in the IRP have provided an opportunity to investigate amine NTs in the CSF of schizophrenics with wide ventricles. These show reductions of both 5HIAA and HVA (which remain highly correlated as in all other populations); the low 5HIAA and HVA are associated with poor response to drugs.

5. Other collaborative studies focused on DA and its metabolites have identified a much greater variance of HVA in plasma of schizophrenics, providing the first approach, of which we are aware, of studying altered regulation of DA in the periphery. Our hope is that this may be an analogue to the abnormal regulation of the NE system found in depressed patients.

Significance to Biomedical Research and to the Program of the Institute:

The major theories about the biological causes of the most prevalent severe psychiatric disorders, depression and schizophrenia, center on monoamine neurotransmitter systems. This project applies sophisticated laboratory assays directly to human studies of monoamine metabolism. Results expand our understanding of the role of norepinephrine in depression and the possible mechanisms of action of antidepressant treatments. New leads suggest that schizophrenia(s) may be subtyped on a biochemical as well as brain imaging basis. The personal and social costs of depression and schizophrenia are great. Insofar as careful clinical research, drawing on basic biochemical techniques, can suggest biological factors in these disorders, specific pharmacologic treatments can be developed and tested in therapeutic trials.

Proposed Course:

We will perform sufficient studies in unipolar and bipolar depressed patients to see if combined measures using CSF, plasma and urine (including Kopin's proposed "correction factor" for CSF contribution to MHPG production) can clearly distinguish subtypes as well as predict treatment response.

We shall systematically evaluate what are the "controls" and work out determinants other than production and release of the plasma concentration of transmitters and their metabolites--such as renal clearance. Furthermore, we shall design additional studies to characterize the conversion rates of NE to variance metabolites so as to interpret new findings.

We will continue to collaboratively study schizophrenic patients focusing on the DA system and its interaction with NE since our preliminary data suggests that real progress may be possible in the biochemical subtyping of this illness.

Publications:

Linnoila, M., Karoum, F. and Potter, W.Z.: High positive correlation between urinary free tyramine excretion rate and "whole body" norepinephrine turnover in depressed patients. Biological Psychiatry 17:1031-1036, 1982.

Scheinin, M. and Linnoila, M.: Acidic amine buffers in electrochemical detection liquid chromatography. Acta Pharmacol. et Toxicol. 51:270-272, 1982.

Linnoila, M., Karoum, F., Cutler, N.R., and Potter, W.Z.: Temporal association between depression-dependent dyskinesias and high urinary phenylethylamine output. Biol. Psychiatry 18:513-516, 1983.

Linnoila, M., Whorton A.R., Rubinow, D.R., Cowdry, R.W., Ninan, P.T. and Waters, R.N.: Cerebrospinal fluid prostaglandin levels in depressed and schizophrenic patients. Arch. Gen. Psychiatry 40:405-406, 1983.

Petrucelli B., Bakris, G., Miller, T., Korpi, E. and Linnoila M.: A liquid chromatographic assay for 5-hydroxytryptophan, serotonin and 5-hydroxyindole-acetic acid in human body fluids. Acta Pharmacol. Toxicol. 51:421-427, 1982.

Chang, W.-H., Scheinin, M., Burns, R.S. and Linnoila, M.: Rapid and simple determination of homovanillic acid in plasma using high performance liquid chromatography with electrochemical detection. Acta Pharmacol. et Toxicol., in press.

Linnoila, M., Ninan, P.T., Scheinin, M., Waters, R.N., Chang, W.-H., Bartko, J. and van Kammen, D.P.: Reliability of norepinephrine and major monoamine metabolite measurements in cerebrospinal fluid of schizophrenic patients. Arch. Gen. Psychiatry, in press.

Linnoila, M., Karoum, F., Miller, T. and Potter, W.Z.: Reliability of urinary monoamine and metabolite output measurements in depressed patients. Am. J. Psychiatry 140:1055-1057, 1983.

Scheinin, M., Chang, W.-H., Jimerson, D.C. and Linnoila, M.: Measurement of 3-methoxy-4-hydroxyphenylglycol in human plasma with high performance liquid chromatography using electrochemical detection. Anal. Biochem. 131:246-253, 1983.

Linnoila, M., Cowdry, R., Lamberg, B. and Rubinow, D.: CSF rT₃ levels in patients with affective disorders. Biol. Psychiatry, in press.

Potter, W.Z., Ross, R.J. and Zavadil, A.P., III: Norepinephrine in the affective disorders. I. Classic biochemical approaches. In The Catecholamines in Psychiatric and Neurologic Disorders, C.R. Lake and M.G. Ziegler (eds.), Butterworth, Inc., Woburn, MA, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00450-09 CP

PERIOD COVERED

October 1, 1982, through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biological rhythms in affective illness

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Basic research in biological rhythms can be related to affective illness with respect to (1) the involvement of disturbed circadian rhythms (24 hour cycles) in the pathophysiology of the illness and (2) the inherent cyclicality of the illness (itself a type of biological rhythm). Indications of circadian rhythm involvement in the illness come from clinical observations: depressive symptoms exhibit a daily rhythm with worsening in the morning and improvement in the evening; the timing of the sleep-wake cycle and the sleep-wake fraction are abnormal. Previous clinical investigations of many physiological and biochemical variables have shown that circadian rhythm phases, amplitudes and waveforms are abnormal in some depressives.

This project is designed to further investigate the behavior of circadian rhythms and manic-depressive cycles in affective patients studied naturalistically and longitudinally. Continuous around-the-clock measurements of wrist motor activity, rectal temperature and sleep EEG are obtained using ambulatory electronic monitoring devices. In some instances circadian rhythms in plasma hormones are measured for 1-3 days via indwelling venous catheters.

Results to date indicate the following circadian rhythm abnormalities in depressed patients: (1) phase instability with both normal and advanced phase positions of the rectal temperature rhythm on different nights in the same patient, (2) abnormal double-length, 48-hour sleep-wake cycles at the time of the switch from depression to mania in rapid cycling manic-depressives, (3) absence of the thyroid stimulating hormone (TSH) circadian rhythm, and failure of sleep deprivation to stimulate TSH secretion in rapid cycling patients. These last findings are particularly interesting in light of other evidence that thyroid impairment may predispose to the rapid cycling form of the illness.

From continuous long-term monitoring of rapid cycling patients we have found (1) additional cases where tricyclic antidepressants markedly accelerated or induced rapid cycling, (2) fundamental independence of manic-depressive and menstrual cycles.

(205)

Other Professional Personnel:

David Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Wallace Duncan	Research Psychologist	CP/NIMH

Project Description:

There is considerable evidence that biological rhythms are involved or are disturbed in affective illness. For example, the course of the illness is itself rhythmic: Depressive symptoms exhibit 24-hour patterns of variation in severity (the classical diurnal variation in mood), and depressions may recur cyclically every few days, weeks or months, or in some cases annually. The sleep-wake cycle is disturbed in depression and mania. Sleep disturbances include altered timing, amount and depth of sleep (these may be considered to represent changes in phase, waveform and amplitude of the sleep-wake cycle). Within depressive sleep the timing of REM sleep is abnormally advanced, possibly reflecting an advance in the phase position of the REM sleep propensity circadian rhythm. In some patients phase, amplitude and waveform of other circadian rhythms, such as rectal temperature, plasma cortisol and urinary potassium have been reported to be abnormal. The fact that experimental alterations in the timing of the sleep-wake cycle relative to other circadian rhythms leads to dramatic clinical state changes in many patients suggests that disturbances in the circadian system play an important role in the pathophysiology of the illness and are not simply epiphenomena.

The purpose of this project is to further document the nature of circadian rhythm disturbance in the illness. The novel feature of this project is its longitudinal approach with continuous around-the-clock monitoring of several variables. This approach permits us to observe the ongoing behavior of circadian rhythms over time and also to observe the long-term cyclic behavior of the illness itself.

Methods:

Patients with major depressive disorder and manic-depressives are observed longitudinally. The majority of patients have a rapid cycling form of the illness, so that it is possible to observe several episodes of depression and mania prospectively and entirely.

Several variables are monitored continuously for weeks or months: wrist motor activity, rectal temperature, sleep EEG and self- and nurses' ratings of mood. In addition, in some cases blood samples are obtained every 30 minutes for 1 to 3 days and subsequently analyzed for various hormones. In a related project the REM sleep propensity circadian rhythm is studied (Project Z01 MH 02204-01 CP).

Findings to date:

1. Motor activity. Wrist motor activity levels are highly correlated with clinical state changes, with activity increased in mania and decreased in depression. Using a discrimination threshold, activity counts are highly correlated with wakefulness, so that the wrist activity monitor can be used to monitor the sleep-wake cycle.

2. Sleep EEG. The timing and amount of sleep changes cyclically with the illness. In many instances the switch from depression to mania is accompanied by the occurrence of one or several double-length, 48-hour sleep-wake cycles. These alternate sleepless nights may play an important role in the spontaneous depression-to-mania switch process since experimental sleep deprivation in such patients can induce depression-to-mania switches. Somewhat similar double-length sleep-wake cycles occur in normal persons when they are isolated from external time cues. Thus 48-hour sleep-wake cycles in patients may arise from a mechanism that is present, but latent, in the normal physiology of the human circadian system.

3. Rectal temperature. Compared with normal controls patients exhibit high night-to-night variability in the timing of their temperature minima. On many nights the temperature rhythm appears to be phase-advanced, as predicted by the phase-advance hypothesis of depression which attributes depressive REM sleep abnormalities to a phase-advance in the REM sleep propensity circadian rhythm.

4. Hormone circadian rhythms. In 3 of 3 rapid cycling manic-depressive women we found the daily variation of pituitary TSH secretion to be absent. Furthermore, sleep deprivation, which normally stimulates TSH secretion, was ineffective. These results provide further evidence of thyroid axis impairment in patients predisposed to the rapid cycling form of the illness.

5. Rhythmic behavior of manic-depressive cycles. In several patients whose manic-depressive cycles appeared to coincide with their menstrual cycles, very long-term observations proved that the two cycles were fundamentally independent. Thus, while rapid cycling is more common in women, the cycles themselves do not arise from the menstrual cycle. In several respects the rhythmic behavior of manic-depressive cycles resembled that of the sleep-wake cycle. Both cycles are characterized by two levels of activation with fairly clearcut switch-over points, by marked lability in period, sometimes with doubling of cycle duration, and by a capacity to interact with or become entrained to other biological or environmental rhythms.

Significance to Biomedical Research and to the Program of the Institute:

1. The wrist activity monitor appears to be a cost effective method for continuously monitoring clinical state changes in manic-depressive illness and in the human sleep-wake cycle. It may prove valuable in the diagnosis and management of affective and sleep disorders, especially in outpatient settings where observations are otherwise quite limited.

2. Abnormalities observed in circadian rhythms suggest that the functioning of a hypothalamic circadian pacemaker, or pathways by which it is entrained to the solar day are disturbed. This hypothesis is currently being investigated by studying the behavior of the circadian pacemakers in patients living in isolation from external time cues (Project Z01 MH 02199-01 CP).

Proposed Course:

A few additional patients will be sought for long-term, longitudinal studies. Much data, especially concerning the possible interrelationship between sleep EEG and temperature, remains to be analyzed.

Publications:

Wehr, T.A.: Circadian rhythm disturbances in depression and mania. In Rhythmic Aspects of Behavior, Brown, F. and Graeber, R.C. (eds.), Lawrence Erlbaum Associates, Hillsdale, New Jersey, pp. 399-428, 1982.

Wehr, T.A., Gillin, J.C., and Goodwin, F.K.: Sleep and circadian rhythms in depression. In Advances in Sleep Research, Vol. 8, Chase, M. (ed.), Spectrum Publications, pp. 195-225, 1983.

Goodwin, F.K., Wirz-Justice, A., and Wehr, T.A.: Evidence that the pathophysiology of depression and the mechanism of action of antidepressant drugs both involve alterations in circadian rhythms. In Advances in Biochemical Psychopharmacology, Vol. 32, Costa, E. and Racagni (eds.), Raven Press, New York, pp. 1-11, 1982.

Wehr, T.A. and Goodwin, F.K.: Introduction. In Biological Rhythms and Psychiatry, Vol. II, Wehr, T.A. and Goodwin, F.K. (eds.), Boxwood Press, California, pp. 1-16, 1983.

Wehr, T.A. and Goodwin, F.K.: Biological rhythms and manic-depressive illness. In Biological Rhythms and Psychiatry, Vol. II, Wehr, T.A. and Goodwin, F.K. (eds.), Boxwood Press, California, pp. 129-184, 1983.

Wehr, T.A., Sack, D.A., Rosenthal, N.E., Duncan, W.D. and Gillin, J.C.: Circadian rhythm disturbances in manic-depressive illness. Federation Proceedings, 42: 2809-2814, 1983.

Rosenthal, N.E., Lewy, A.J., Wehr, T.A., Kern, H.E. and Goodwin, F.K.: Seasonal cycling in a bipolar patient. Psychiat. Res., 8: 25-31, 1983.

Rosenthal, N.E., Sack, D.A. and Wehr, T.A.: Seasonal variation in affective disorders. In Biological Rhythms in Psychiatry, Vol. II, Wehr, T.A. and Goodwin, F.K. (eds.), Boxwood Press, California, pp. 185-202, 1983.

Wehr, T.A.: Biological rhythms and manic-depressive illness, in Ballenger, J.C., Post, R.M. (eds.), Neurobiology of the Mood Disorders, Williams & Wilkins, Baltimore/London, in press, 1983.

Wehr, T.A., Wirz-Justice, A., Goodwin, F.K.: Circadian rhythm disturbances in affective illness and their modification by antidepressant drugs, in Davis, J.M., Maas, J.W. (eds.), Affective Disorders. American Psychiatric Press, Inc. pp. 333- 346, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02196-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Causes of the delayed sleep phase syndrome		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: .5	OTHER: .5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Delayed sleep phase syndrome (DSPS) is a type of sleep disorder</u> <u>characterized by marked difficulty falling asleep at night and marked</u> <u>difficulty waking up in the morning. When sleep occurs, it is normal in</u> <u>content and duration. Thus DSPS is a disorder of the timing, not the quality,</u> <u>of sleep. It is hypothesized that DSPS arises from a disturbance in the</u> <u>biological clock that regulates the timing of sleep. The normal human sleep-</u> <u>wake cycle is controlled by a biological clock that in the absence of external</u> <u>influences generates a 25-hour rhythm. The timing of this circadian rhythm</u> <u>ordinarily is controlled by periodic stimuli in the environment that have the</u> <u>properties of time cues (zeitgebers), so that it maintains (1) a period of 24</u> <u>hours and (2) a characteristic phase relationship to the day-night cycle (i.e.,</u> <u>sleep occurs every 24 hours, and mainly at night). Throughout biology light</u> <u>(e.g., sunrise, sunset) is the most important zeitgeber. In theory DSPS could</u> <u>be caused by (1) an abnormally slow intrinsic rhythm of the sleep-wake cycle</u> <u>pacemaker (e.g., 25.5-26 hours) or (2) an abnormal response to zeitgebers,</u> <u>such as light.</u> </p> <p> The purpose of this project is to investigate these two possible causes of DSPS: (1) the intrinsic rhythm of DSPS patients will be measured by asking them to live for at least two weeks in isolation from external time cues in special experimental rooms on the 4-West research unit. During the study motor activity and rectal temperature will be monitored continuously by computer. Circadian rhythm periods significantly longer than 25 hours would implicate a slow pacemaker as a cause of DSPS; (2) DSPS patients' hypothalamic sensitivity to light will be measured by investigating the degree to which nocturnal secretion of melatonin can be suppressed by varying intensities of light. Abnormal sensitivity to light would indirectly implicate altered sensitivity to zeitgebers as a cause of DSPS. Responses to light will also be evaluated using conventional optical and electrophysiological techniques. </p>		

Other Professional Personnel:

Norman E. Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
David Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Steven James, M.D.	Clinical Associate	CP/NIMH

Project Description:

Delayed sleep phase syndrome (DSPS) is a type of sleep disorder characterized by initial insomnia, followed by normal sleep and difficulty awakening. It is a disorder of the timing of sleep and is thought to arise from a disturbance in the biological clock that controls the sleep-wake cycle. Theoretically a phase-angle abnormality in the sleep-wake cycle could arise from at least two causes: (1) an abnormally slow intrinsic rhythm of the circadian pacemaker or (2) abnormal insensitivity to the phase-setting properties of an environmental time cue (zeitgeber), such as light. This project is designed to investigate these two possible causes of DSPS.

Selection of Subjects:

DSPS patients will be screened and selected on the basis of the following criteria:

- (a) initial insomnia (sleep onset after 2 AM)
- (b) normal maintenance of sleep
- (c) difficulty arising in the morning

These features will be confirmed by polysomnography.

Methods:

(1) Measurement of the period of the intrinsic rhythm of the circadian pacemaker in DSPS. Patients with DSPS will live in special isolation units on the 4-West research unit from which external time cues have been excluded. In these conditions their sleep-wake cycle and circadian rhythms can be expected to persist and "free-run" with a period that differs from 24 hours and reflects the intrinsic period of the circadian pacemaker. Normally this period is about 25 hours. We hypothesize that the period in DSPS will be longer than 25 hours. Circadian period estimates will be derived from continuous on-line computer monitoring of motor activity, rectal temperature and behavioral events, and sleep EEG recordings. Phase reference points for period estimates will include temperature maxima and minima, and wake and sleep onset. Periodogram and Fourier analysis will also be used.

(2) Measurement of sensitivity to light. DSPS patients' responses to light will be evaluated with conventional neuro-ophthalmological tests (dark adaptometry, color vision, electro-retinogram) and suppression of nocturnal melatonin (MT) secretion by bright artificial light. Patients' responses to light suppression of MT will be evaluated with a fluence response curve generated by exposing them to varying intensities of light and measuring the degree of MT suppression as described in Project Z01 MH 02200-01 CP. We hypothesize that patients will be abnormally insensitive to light acting via this hypothalamic mechanism.

Findings to date: Project just beginning.

Significance to Biomedical Research and to the Program of the Institute:

1. DSPS is a significant public health problem. 15-20% of patients evaluated in sleep disorders clinics have DSPS.
2. Sedative-hypnotic abuse and dependence are also significant public health problems. Such patients are vulnerable to physical dependence on sedative-hypnotic drugs, which have no real therapeutic value in the syndrome. Any progress in understanding the pathophysiologic mechanism underlying the disorder may lead to new types of treatment and, possibly, prevention.
3. Clarification of the underlying pathophysiologic mechanism of DSPS would dramatically demonstrate the relevance of principles drawn from basic research in circadian physiology to clinical problems such as insomnia.

Projected Course: Patients will be recruited this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02197-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Treatment of delayed sleep phase syndrome with light		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: .5	OTHER: .5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Delayed sleep phase syndrome (DSPS) is a sleep disorder characterized by difficulty falling asleep until 2 AM or later, normal sleep after sleep onset, and difficulty arising in the morning before 9 AM or later. DSPS is a disorder of the timing of sleep, not its quality, and as such is thought to arise from a disturbance in the biological clock in the brain that controls the <u>sleep-wake cycle</u>. Normally, the clock mechanism responds to periodic time cues in the <u>environment</u> (zeitgebers) in such a way that sleep occurs at night. In DSPS the timing of the clock mechanism is shifted later (delayed) so that sleep can only occur after 2 AM or later.</p> <p>It is well documented in many species that light (e.g., dawn and dusk) is the zeitgeber that maintains the proper timing, or phase control, of biological clocks. Experiments are currently under way in this branch to establish whether or not light is an important zeitgeber in human beings (Project Z01 MH 02198-01 CP). The goal of this project is to determine whether or not delayed sleep phase syndrome can be treated using bright artificial light administered at a critical time of day. Based on animal studies we hypothesize that light given daily in the morning will gradually advance the timing of the sleep-wake cycle to a more nearly normal schedule in DSPS. We hypothesize that light administered in the evening will have no, or an opposite, effect. Morning and evening light will be administered to DSPS patients in a randomly sequenced cross-over design. Evening light appears to be a credible sham control treatment.</p> <p>All patients entering the study will be carefully evaluated for the presence of other sleep, psychiatric and medical disorders. A special effort will be made to find possible predisposing or contributing factors, since little is known of the cause of DSPS. The daily sleep-wake cycle will be monitored longitudinally with <u>wrist activity monitors</u> developed at the NIMH.</p>		

Other Professional Personnel:

Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Norman E. Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Steven James, M.D.	Clinical Associate	CP/NIMH

Project Description:

Delayed sleep phase syndrome (DSPS) is a sleep disorder characterized by difficulty falling asleep until 2 AM or later, normal sleep after sleep onset, and difficulty arising in the morning before 9 AM or later. DSPS is a disorder of the timing of sleep, not its quality, and as such is thought to arise from a disturbance in the biological clock in the brain that controls the sleep-wake cycle. Normally, the clock mechanism responds to periodic time cues in the environment (zeitgebers) in such a way that sleep occurs at night. In DSPS the timing of the clock mechanism is shifted later (delayed) so that sleep can only occur after 2 AM or later.

It is well documented in many species that light (e.g., dawn and dusk) is the zeitgeber that maintains the proper timing, or phase control, of biological clocks. Experiments are currently under way in this branch to establish whether or not light is an important zeitgeber in human beings (Project Z01 MH 02198-01 CP). The goal of this project is to determine whether or not delayed sleep phase syndrome can be treated using bright artificial light administered at a critical time of day. Based on animal studies we hypothesize that light given daily in the morning will gradually advance the timing of the sleep-wake cycle to a more nearly normal schedule in DSPS. We hypothesize that light administered in the evening will have no, or an opposite, effect. Morning and evening light will be administered to DSPS patients in a randomly sequenced cross-over design. Evening light appears to be a credible sham control treatment.

Methods:

Potential subjects will be recruited through the media. They will be carefully screened to exclude other sleep disorders such as sleep apnea, narcolepsy, depression, nocturnal myoclonus, etc. Criteria for admission to the protocol are as follows:

- (1) difficulty falling asleep before 2 AM;
- (2) normal sleep after sleep onset;
- (3) difficulty arising in the morning;
- (4) tendency to sleep late on weekends to compensate for sleep loss;
- (5) best functioning in the evening or at night.

Sleep patterns will be documented in three ways:

- (1) several nights' sleep EEG recordings;
- (2) daily sleep logs using OPSCAN forms developed in the CPB;
- (3) continuous monitoring of wrist motor activity using an ambulatory device developed at NIMH-IRP.

Circadian rhythm pacemakers' phase position will be estimated by measuring serum melatonin (MT) levels each hour for 24 hours.

Light treatment is as follows:

All treatments will involve fluorescent day-light spectrum lights of sufficient intensity to suppress nocturnal MT secretion (>2000 lux). In the absence of any other measure hypothalamically-mediated MT suppression seems most likely to reflect the threshold for other hypothalamic effects of light, such as phase control of the circadian pacemaker.

Subjects will be randomly assigned either to morning or evening light, then crossed over to the other condition. Patients who respond will be crossed over only if they relapse after withdrawal of effective treatment.

Light treatments will last one hour each day. Patients will sit 3 feet away from the light source (8 fluorescent bulbs) while engaged in reading or other sedentary activities. Morning light will be administered at progressively earlier times beginning at the patient's usual wake-up time. Evening light will be administered progressively later after dinner. The goal of the treatment is to determine whether light can shift the timing of the sleep-wake cycle to a more nearly normal time.

Findings to date: Project is just beginning.

Significance to Biomedical Research and to the Program of the Institute:

1. DSPS is a significant public health problem, accounting for 15-20% of visits to sleep disorders clinics.

2. Sedative-hypnotic drug dependence is a significant public health problem; DSPS patients are prone to drug dependence. Development of an effective non-drug treatment would relieve suffering and prevent drug dependence.

3. Successful treatment of DSPS would provide further evidence that the timing of the human biological clock is controlled by bright light (e.g., sunlight). Light could be used to treat other circadian rhythm disturbances arising from shift work and jet lag, for example.

4. Successful treatment of DSPS with light would establish an important link between a disease and a major area of basic research concerning the effects of light on the circadian system.

Proposed Course:

If light can be shown to be effective in the treatment of DSPS, the next logical step would be to substitute for light drugs which are known to mimic the effects of light on the hypothalamus (nicotinic agonists). In this case the therapeutic effect would depend on the timing of the drug.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02198-01 CP

PERIOD COVERED

October 1, 1982, through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effect of light on free-running human circadian rhythms

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Psychobiology Branch

SECTION

INSTITUTE AND LOCATION

NIMH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

All species exhibit daily cycles in physiology and behavior called circadian rhythms. For example, in humans core body temperature oscillates between an afternoon high and a nighttime low. Circadian rhythms are generated by a pacemaker located in the hypothalamus. In isolation from external time cues (zeitgebers), the intrinsic period of this pacemaker is about 25 hours. Ordinarily the pacemaker responds to zeitgebers in such a way that it becomes entrained to the 24-hour solar day. In most species light (dawn, dusk) is the most important zeitgeber. The phase response to light of the circadian pacemaker is such that (1) its period becomes 24 hours and (2) it adopts a characteristic phase position relative to the zeitgeber (e.g., sleep occurs once every 24 hours and principally at night).

The effect of light pulses administered to animals free-running in constant conditions depends on when in the circadian cycle the pulse is presented. Pulses near subjective morning produce phase advances, near subjective evening delays, and during subjective daytime no effect. These responses can be used to generate a phase-response curve which shows direction and magnitude of phase shift as a function of subjective time of pulse presentation.

The purpose of this project is to generate a phase response curve to light for the human circadian system. Using suppression of pineal melatonin secretion as a marker, we previously found that the human hypothalamus is relatively insensitive to light. Healthy subjects living for two weeks in constant dim light are exposed to a single 6-hour pulse of bright artificial light (3000 lux) administered at different subjective times in different subjects. Circadian rhythms are monitored longitudinally in rectal temperature, wrist motor activity, sleep EEG, and behavioral events. Shifts in these rhythms induced by light are recorded.

The normal human phase response curve is expected to prove valuable in understanding and treating circadian rhythm disturbances in depression, mania, insomnia (delayed sleep phase syndrome), jet lag and shift work.

Other Professional Personnel:

David A. Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Gerard Groos, Ph.D.	Fogarty Associate	CP/NIMH
Wallace Duncan	Research Psychologist	CP/NIMH
Norman Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH

Project Description:

All species exhibit daily cycles in physiology and behavior called circadian rhythms. For example, in humans core body temperature oscillates between an afternoon high and a nighttime low. The daily sleep-wake cycle is another example. Circadian rhythms are generated by a pacemaker located in the hypothalamus. In isolation from external time cues, or zeitgebers, the free-running or intrinsic period of this pacemaker is about 25 hours. Ordinarily the pacemaker responds to zeitgebers in such a way that it becomes entrained to the 24-hour solar day and its period becomes 24 hours. In all species light (dawn, dusk) is the most important zeitgeber. The phase response to light of the circadian pacemaker is such that (1) its period becomes 24 hours and (2) it adopts a characteristic phase position relative to the zeitgeber (e.g., sleep occurs once every 24 hours and principally at night).

Light pulses administered to animals free-running in constant conditions induce a phase advance, phase delay or no effect depending on when in the circadian cycle the pulse is presented. Pulses near subjective morning produce advances, near subjective evening delays, and during subjective daytime no effect. These responses can be used to generate a phase-response curve which shows direction and magnitude of phase shift as a function of subjective time of pulse presentation.

The purpose of this project is to generate a phase response curve to light for the human circadian system. Using suppression of pineal melatonin secretion as a marker we previously found that the human hypothalamus is relatively insensitive to light. Therefore bright artificial light pulses will be used in the experiment.

Methods:

Healthy subjects will live isolated from relevant external time cues in special experimental rooms on the 4-West research unit. Subjects will live in constant dim light for 2 weeks except for one 6-hour period when they will be exposed to bright fluorescent light (>3000 lux). Light pulses will be administered on different occasions at four equispaced intervals relative to the subjects' sleep-wake cycle (1 pulse per subject). Sleep deprivation without light will be used to control for sleep deprivation caused by certain light pulses. Circadian rhythms in wrist motor activity, rectal temperature, sleep EEG and behavioral events will be monitored continuously partly by on-line computer. Phase shifts induced by light will be measured as follows: period and phase position of the circadian rhythm will be computed and compared before and after the exposure to the light pulses. Magnitude and direction of phase shift will be determined by comparing the expected phase projected from baseline

measurements to the actual phase after the light pulse. A phase response curve will be generated by expressing magnitude and direction of phase shift as a function of the time during the sleep-wake cycle pulses were presented. Phase reference points for these calculations will include sleep onset, wake onset, temperature minimum and temperature aerophase (peak of least squares fitted cosine).

Findings to date:

This is an ambitious project. So far 4 subjects have been studied in baseline (unpulsed) conditions for 2 weeks each in order to perfect the experimental conditions and equipment necessary to perform the study.

Significance to biomedical research and to the program of the Institute:

It is of fundamental importance to determine whether or not light controls the timing of the human biological clock, and to determine what principles govern this control. With a phase response curve for light, animal studies have shown that it is possible to predict most behaviors of the circadian system under conditions of entrainment to a light-dark cycle. The phase response curve predicts the range of entrainment (range of zeitgeber periods to which the system can be entrained) and the phase position of the system relative to the zeitgeber. It is expected that this kind of information can lead to the rational use of bright artificial light in the treatment or prevention of circadian rhythm disturbances in depression, insomnia (delayed sleep phase syndrome), jet lag and shift work.

Projected Course:

If we are successful in generating a human phase response curve for light, we will subsequently attempt to generate a fluence response curve for the phase response; that is, we will study the degree of phase shift as a function of light intensity. We will also attempt to mimic the effects of light with pharmacological agents as has been done in animals. This experiment could lead to novel pharmacological treatments for circadian rhythm disorders outlined above.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02199-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Circadian rhythms in affective disorder patients isolated from time cues		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (if any) Research Services Branch, NIMH		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION 		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.0	PROFESSIONAL: 1.0	OTHER: 4.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Abnormalities of phase, amplitude and waveform of circadian rhythms have been reported in depression and in manic-depressive illness. Abnormalities of the circadian system are thought to play an important role in the illnesses because experimental manipulations of sleep and circadian rhythms are capable of altering clinical state.</p> <p>The human circadian system has been mathematically modeled by a two process or two oscillator system. Depressive sleep abnormalities and the therapeutic effect of various sleep manipulations have also been modeled by changing certain parameters in these mathematical models, such as the intrinsic period of a circadian oscillator or the rates of accumulation and discharge of a sleep factor. We are testing these and other predictions of the models by studying circadian rhythms in patients while they live in isolation from all external time cues in special rooms on the 4-West research unit. Patients stay in the rooms while rectal temperature, wrist motor activity, sleep EEG and behavioral events are continuously monitored by computer. In these conditions circadian rhythms "free-run" according to their own intrinsic period, which is normally about 25 hours.</p> <p>To date four affective patients have been studied for one month each. In one bipolar patient who switched from depression to mania shortly after beginning the experiment, the free-running period was abnormally short (less than 24 hours). Such an abnormally fast circadian pacemaker could explain phase-advanced circadian rhythms under conditions of entrainment to external time cues. A manic patient showed internal desynchronization of sleep and temperature circadian rhythms with a <u>sleep-wake cycle period</u> of 17 hours. The circadian period in two other cases was normal. All cases showed markedly abnormal ratios of sleep to wakefulness during the experiments, indicating that changes in timing and amount of sleep in depression are fundamentally endogenous and do not depend on some interaction with the environment.</p>		

Other Professional Personnel:

David A. Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Wallace Duncan	Research Psychologist	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Norman E. Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Bruce Smith	Electronic Engineer	RSB/NIMH

Project Description:

Several types of circadian rhythm abnormalities have been reported in affective illness:

- (1) phase-advanced circadian rhythms in many variables;
- (2) decreased amplitudes of circadian rhythms, especially decreased minimum amplitude of temperature (higher nocturnal minima).
- (3) instability in phase of circadian rhythms, with large day-to-day variation;
- (4) decreased or increased sleep/wake ratios with shifts in the timing of wake and/or sleep onset.
- (5) double-length, 48-hour (circabidian) sleep-wake cycles.

Certain experiments suggest that changes in the circadian system play a causal role in the illness. Manipulations of the timing of the sleep-wake cycle relative to the timing of their circadian rhythms have marked and immediate antidepressant effects in about 50% of cases. These manipulations include partial sleep deprivation in the second half of the night (effective) versus the first half of the night (ineffective), and six hours phase advance of the sleep-wake cycle.

In order to understand the behavior of the human circadian system, it is necessary to monitor circadian oscillations that are free of all external influences. In conditions of temporal isolation in rooms from which all external time cues have been removed, circadian rhythms free-run according to the intrinsic period of their driving pacemaker (usually about 25 hours). In these conditions it can sometimes be seen that the human circadian system is composed of two oscillating subsystems, which become dissociated with autonomic circadian rhythms (e.g., temperature) exhibiting a circadian period of about 25 hours, and the sleep-wake cycle exhibiting non-circadian periods much shorter or much longer than 24 hours.

Two similar mathematical models have been developed to describe these behaviors of the human circadian system. In both models an oscillator with an intrinsic period near 25 hours is considered to control autonomic circadian rhythms such as that of temperature. The models differ slightly with regard to the generation of the sleep-wake cycle. In the "2 oscillator model" the sleep-wake cycle is considered to be driven by a second oscillator coupled to the first. The sleep-wake cycle oscillator is weaker and has a more labile period

which may extend or shorten considerably relative to 25 hours. In the "2 process model" the sleep-wake cycle is considered to arise from the buildup and breakdown of a hypothetical sleep substance. The autonomic circadian oscillator sets a changing threshold for the sleep-inducing effect of substance "S" and for the waking that occurs when S is exhausted.

Depression has been described in terms of these two models. Phase advanced circadian rhythms are hypothesized to result from either (1) an abnormal sensitivity to zeitgebers (time cues) such as light that control the timing or phase position of circadian rhythms or (2) an abnormally short intrinsic period of the autonomic circadian oscillator. The intrinsic period of this oscillator can only be measured when patients' circadian rhythms are monitored while they live in isolation from external time cues. In the two process model, sleep abnormalities and mood disturbances in depression are considered to arise from a reduced rate of accumulation of substance S during wakefulness. This model predicts that in temporal isolation very long sleep-wake cycles with internal desynchronization are likely to occur.

Our goal in this project is to test predictions of the two models by studying the behavior of circadian rhythms in patients living in isolation from external time cues. Predictions of the models are: (1) in depressed patients with phase-advanced circadian rhythms, abnormally short intrinsic periods of the autonomic circadian rhythms will be observed; (2) in depressed patients with characteristic sleep disturbances, internal desynchronization with very long sleep-wake cycles will be observed.

Methods

Patients are selected for study on the basis of R.D.C. diagnosis of depression or mania and their suitability for living in isolation (low suicide risk, etc.) They live in special rooms on the 4-West research ward from which relevant external time cues have been removed. Windows are blocked. Rooms are entered through a vestibule that functions as a lock. Meals are served on request. Personnel have contact with the patients on random schedules.

Rectal temperature, wrist motor activity, sleep EEG and behavioral events are monitored continuously, partly by on-line computer. Nurses make observations every 30 minutes via infrared video monitors.

Patients are instructed to go to sleep, wake up and eat meals whenever they wish.

Circadian rhythm periods and phases are determined by several mathematical techniques, including linear regression, Fourier analysis, periodogram and least squares cosine fitting.

Findings to date:

Four patients have been studied, 3 bipolar, 1 unipolar. All had characteristic sleep abnormalities (short REM latency, long first REM period, low delta sleep) prior to entering the study. All remained in temporal isolation for one month. One unipolar (A) patient was depressed throughout the study. One

patient (B) who was depressed switched into mania after five days of temporal isolation. One patient (C) rapidly cycled between mania and depression before and during isolation, and one patient (D) was manic throughout the study after treatment with clorgyline, a Type A MAOI (other patients were drug free).

Patients A and C remained internally synchronized and had normal circadian periods (near 25 hours).

Patient B, after switching into mania, exhibited an abnormally short circadian period less than 24 hours.

Patient D showed internal desynchronization with shortening of the sleep-wake cycle.

All four patients showed marked abnormalities in the ratio of sleep to wake durations.

Hypothesis #1 is supported in one patient.

Hypothesis #2 is supported in no patients.

Significance to Biomedical Research and to the Program of the Institute:

1. This project is aimed at understanding fundamental pathogenic mechanisms in depression. Insight into these mechanisms could lead to new forms of treatment. For example, drugs or methods that alter the timing of biological clocks might be devised and explored.

2. The goal of this project is to rigorously test hypotheses about the circadian system in depression in the only conditions in which they can be tested, temporal isolation. The project makes use of principles drawn from 20 years of study of the normal human circadian system in temporal isolation.

Proposed Course:

Additional patients will be sought and studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02200-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Light suppression of nocturnal human melatonin secretion		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas A. Wehr, M.D., Acting Chief, Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.25	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Melatonin (MT) is secreted by the pineal gland (PG) almost exclusively at night. Our previous work has shown that MT is present in humans and that its secretion can be suppressed with bright artificial light (>2000 lux). This effect of light is presumed to be mediated by neural pathways connecting the retina to the PG via the hypothalamus. At the hypothalamic level the effect of light is thought to be mediated by <u>nicotinic cholinergic receptors</u>. Suppression of MT is presently the only index of hypothalamic sensitivity to light. We have shown that humans have a high threshold for light-MT effects compared with experimental animals. Ordinary artificial light, for example, is ineffective in humans.</p> <p>Abnormal hypothalamic sensitivity to light may be an important trait and pathogenic mechanism in <u>manic-depressive illness</u> (Project Z01 MH 02199-01 CP), <u>seasonal affective disorder</u> (Projects Z01 MH 02205-01 CP and Z01 MH 02206-01 CP) and <u>delayed sleep phase syndrome</u> (Projects Z01 MH 02196-01 CP and Z01 MH 02197-01 CP).</p> <p>The purpose of this project is (1) to standardize the light-MT suppression test (LMST), (2) to identify sources of variance in the LMST (age, sex, prior sleep or waking, and prior exposure to light), and (3) to investigate hypothalamic sensitivity to light using the LMST in the disorders outlined above. We will standardize the administration of light by using a xenon light source, infrared and ultraviolet filters, fiberoptic light guides, and ganzfeld-type goggles. Intensities of light can be varied using neutral density filters. If desired, monochromatic filters can also be used. Light will be administered for one hour. Blood samples will be obtained before and at the end of light administration. Using these methods we will develop a <u>fluence-response curve</u> for the LMST in which degree of MT suppression is expressed as a function of log light intensity (watts/cm²).</p>		

Other Professional Personnel:

Steven James, M.D.	Clinical Associate	CP/NIMH
Barbara Parry, M.D.	Clinical Associate	CP/NIMH
David Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Norman Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH

Project Description:

Melatonin (MT) is formed from serotonin by N acetyl transferase (NAT) and 5-hydroxy-0-methyl transferase (5H10MT) in the pineal gland (PG) and is released into the blood and CSF. Melatonin secretion is stimulated by neural impulses originating in the suprachiasmatic nucleus (SCN) of the hypothalamus and transmitted through sympathetic outflow to the superior cervical ganglion (SCG) and thence to the PG. In all species MT is secreted almost exclusively at night. Its cyclic nocturnal secretion is driven by the clocklike behavior of the SCN, a circadian rhythm pacemaker. Nocturnal secretion of MT can be suppressed by exposure to light. Light acts on the SCN via the retinohypothalamic tract (RHT). Intraventricular carbachol, a cholinergic nicotinic agonist mimics the effects of light on the SCN; therefore the RHT-SCN innervation is thought to be cholinergic. Cholinergic synapses also occur in the SCG. The SCG innervates the PG via noradrenergic fibres and post-synaptic beta receptors. MT secretion is stimulated by beta agonists and inhibited by beta blockers.

Our previous work, using a gas chromatograph mass spectrometric (GCMS) assay, has shown that (1) MT is present in human blood, (2) is secreted mostly at night, (3) is suppressed by beta blockers, (4) is suppressed by bright artificial light. We showed that the MT rhythm is synchronized poorly or not at all with the day-night cycle in blind persons. We also found that manic-depressive patients appear to be supersensitive to the MT suppressing effects of light, regardless of clinical state.

The hypothalamus-pineal axis has a very high threshold for suppression by light in humans compared with animals (2000 lux versus 10-50 lux). The human threshold is such that artificial light ordinarily present in home and work environments has little effect.

There is theoretical and empirical support for the hypothesis that abnormal hypothalamic sensitivity to light is a trait and a pathogenic mechanism in manic-depressive illness (Project Z01 MH 02199-01 CP), seasonal affective disorder (Projects Z01 MH 02205-01 CP and Z01 MH 02206-01 CP) and delayed sleep phase syndrome (Projects Z01 MH 02196-01 CP and 02197-01 CP).

The purpose of this project is (1) to standardize the light MT suppression test (LMST), (2) to identify sources of variance in the LMST, and (3) to use the LMST to investigate hypothalamic sensitivity to light in the disorders outlined above.

Methods:

Light will be administered in a quantitative manner using a xenon lamp, infrared and ultraviolet filters, fiberoptic light guides and ganzfeld-type

goggles. Intensity of light will be varied using neutral density filters. Light will be administered for one hour from 1 AM to 2 AM. Blood samples will be obtained from subjects before and after exposure to light.

Using this method a fluence response curve for the LSMT will be obtained in which degree of suppression is expressed as a function of log light intensity (watts/cm²).

The following possible sources of variance will be investigated: height, weight, age, sex, phase of menstrual cycle, prior sleeping and waking, and prior exposure to light.

Subsequently the various diagnostic groups will be investigated.

Findings to date: Project to begin shortly.

Significance to Biomedical Research and to the Program of the Institute:

1. Preliminary findings with the LMST indicate abnormal hypothalamic sensitivity to light in manic-depressive illness. To have confidence in these results, the studies must be repeated using a more sensitive and quantitative method of administering light.

2. Abnormal hypothalamic sensitivity to light, if present in delayed sleep phase syndrome, manic-depressive illness or seasonal affective disorder, could have pathogenic significance.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02201-01 CP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Early versus late partial sleep deprivation in the treatment of depression		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David A. Sack Chief, Clinical Research Unit		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Various manipulations of the sleep wake cycle have antidepressant effects in endogenously depressed patients. <u>Total sleep deprivation and partial sleep deprivation</u> in the second half of the night induce remissions during the following day. Also, advancing the sleep period several hours earlier than its usual time without a decrease in the length of the sleep period (<u>phase-advance of the sleep wake cycle</u>) also may induce clinical remissions.</p> <p>Some studies show that the timing of various <u>circadian rhythms</u> and REM sleep appears to be shifted to an abnormally early time in depression. In patients with phase-advanced circadian rhythms, both the phase-advance treatment and partial sleep deprivation in the second half of the night, shifts sleep earlier, restoring a more normal relationship between sleep and the other circadian rhythms. If the timing of sleep and not the duration is the critical factor in the sleep deprivation response, then partial sleep deprivation in the first half of the night should not have therapeutic effects.</p> <p>In this study we have examined the relative efficacy of partial sleep deprivation (PSD) in the first half of the night (E = early) and PSD in the second half of the night (L = late) in a randomized crossover design. All depressed patients underwent baseline sleep, temperature, activity, (24 hour) U.F.C., TRH stimulated TSH response and 24 hour urinary collections for norepinephrine, serotonin and their metabolites in order to investigate the relationship between the sleep deprivation response and the current biochemical and neuroendocrine hypotheses of depression.</p> <p>Of 12 drug free depressed patients (6 UP, 6 BP) six responded to PSD. All six improved on the PSD-L condition. None responded when kept awake in the first half of the night. Results of this study support the hypothesis that the internal phase relationship between sleep and other circadian rhythms is critical to the antidepressant response of PSD. In addition the response to PSD was not restricted to the first night. Improvement was sustained through a second night of PSD and a night of recovery sleep.</p>		

Other Professional Personnel:

Thomas A. Wehr, M.D.	Acting Chief	CP/NIMH
Norman E. Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
Barbara A. Parry, M.D.	Clinical Associate	CP/NIMH
Wallace B. Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH

Project Description:

The timing of various circadian rhythms appears to be shifted to an abnormally early time in depression, raising the possibility that the timing of sleep relative to circadian rhythms (their internal phase relationship) is a pathogenic factor in affective illness. The antidepressant effects of partial sleep deprivation may, therefore, depend on the time at which sleep occurs. The objectives of this study are:

- 1) To determine the relative efficacy of sleep deprivation in the first half versus the second half of the night.
- 2) To describe the diagnostic, biochemical neuroendocrine and psychophysiological predictors of the sleep deprivation response.
- 3) To determine the effects of sleep deprivation on neurotransmitters whose function is thought to mediate other antidepressant responses, and sleep EEG and neuroendocrine systems the function of which is disturbed in depression.

Methods:

Patients were included if they met RDC criteria for a major affective disorder, and had been free of all psychotropic medications for at least two weeks. All subjects underwent a baseline evaluation which included: 1) EEG recorded sleep 2) 24 hour rectal temperature monitoring 3) 24 hour urine collection for norepinephrine and its metabolites and urinary free cortisol 4) TRH stimulated TSH study. EEG recorded sleep and 24 hour urine collections were repeated daily throughout the study. Following two baseline days each subject was randomized to partial sleep deprivation (PSD) in the first half of the night (sleeping from 2 a.m. to 7 a.m.) or PSD in the second half of the night (sleeping from 9 p.m. to 2 a.m.). Patients were kept on the PSD schedule for two successive nights and then returned to a normal sleep schedule (11 p.m. to 7 a.m.).

Improvement in mood was assessed on a nurses global assessment scale and a visual analogue self rating scale (100 mm line) obtained every two hours while awake through all five days of the study. In addition a standardized video taped interview was performed every four hours during the same period to be used for "blind" ratings.

Patients who relapsed following several recovery nights sleep, were crossed over to the other PSD condition after the baseline studies had been repeated.

Findings:

Twelve subjects so far have completed the crossover design. Of these, six experienced a significant response to PSD. All six responded to PSD in the second half of the night as measured by nurses' ratings and self ratings. There was no improvement when PSD occurred in the first half of the night. In our subjects clinical improvement was sustained following a second night of PSD and a night of recovery sleep.

Our preliminary findings suggest that PSD is only effective when patients are awake in the second half of the night. PSD in the first half of the night appears to be a useful sham procedure to control for placebo effects. Repeated nights of PSD may prevent the high rate of relapse seen in total sleep deprivation experiments.

Significance to Biomedical Research and to the Program of the Institute:

1. Our findings indicate that PSD in the first half of the night can be a plausible sham procedure in future clinical studies. This is important because previous studies of sleep deprivation were hampered by the lack of an adequate control.

2. Sleep deprivation is one of three effective treatments for severe depression (antidepressants and ECT being the other two) but its clinical application has been limited by the brevity of its response. It appears from this limited sample that PSD can produce marked and sustained effects and that its clinical application and its mechanism of action require further investigation.

3. Partial sleep deprivation provides a clinical bridge between circadian and biochemical formulations of depression. The effects of PSD on neuro-transmitter function may provide important insights into the pathophysiology of depression. Studies of neuronal function during the second half of the night may suggest new pharmacologic models for antidepressant medications.

4. This experiment provides further evidence that the timing of sleep is critical to the maintenance of the depressive syndrome in certain individuals. It appears that the second half of the night corresponds to a "critical phase" when sleep or wakefulness exerts a powerful effect on the clinical state of depressed patients. This suggests that the pathophysiology of depression can only be understood when patients are studied from 2 a.m. to 7 a.m., a time when they have seldom been studied in the past.

Proposed Course:

In the following year we will extend our study to an additional 15 subjects. This will enable us to confirm our preliminary impressions on the temporal specificity of the PSD response. Additional subjects are necessary for the interpretation of the neuroendocrine and neurochemical studies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02202-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical features of seasonal affective disorder (SAD)		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Norman E. Rosenthal, M.D., Staff Psychiatrist, CPB, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 0.6	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Epidemiological studies have shown a strong relationship between the incidence of suicides, affective episodes and the seasons. In order to understand this association, we have studied patients who regularly become depressed at a certain time of year. Most of these patients become depressed in winter and recover or become hypomanic in spring or summer. We have studied 79 such patients with Seasonal Affective Disorder (SAD). Most are women with an onset of illness in their twenties. During depressions they become lethargic, overeat, oversleep, gain weight and withdraw from friends and family. Although they rarely require hospitalization and hold their jobs, their level of functioning deteriorates. Most SAD patients note that their depressions improve when they travel south in the winter.</p> <p>There is a marked annual rhythm in season of birth of the children of these patients, suggesting that this condition may be a human equivalent of seasonal rhythms of reproduction in animals.</p> <p>There is a strong negative correlation between the timing of depressive symptoms and day length and environmental temperature. A study of bipolar patients revealed that they have a pattern of seasonal vulnerability to depression intermediate between that of SAD patients and normal volunteers. Patients with SAD more frequently have premenstrual mood problems than the general population.</p> <p>Ongoing longitudinal studies involving computer-scanned daily ratings and other measures are aimed at further describing the relationship between climatic variables and mood, sleep and behavior.</p>		
(233)		

Other Professional Personnel:

David A. Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Steven P. James, M.D.	Clinical Associate	CP/NIMH
Barbara L. Parry, M.D.	Clinical Associate	CP/NIMH
Wallace B. Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Thomas A. Wehr, M.D.	Acting Chief	CP/NIMH
Elliot S. Gershon, M.D.	Chief, Section on Psychogenetics	BP/NIMH
John I. Nurnberger, M.D.	Medical Officer (Psychiatry)	BP/NIMH
David R. Rubinow, M.D.	Chief, Unit on Peptide Studies	BP/NIMH

Project Description:

There is a well established association between seasonal changes and the incidence of affective episodes and suicides in the population. Seasonal variations in several neurotransmitters and hormones considered to be relevant to the pathophysiology of depression have been described. Although these studies have shown correlations between mood states and the seasons, their design has not allowed for a clear understanding of the mechanism of these seasonal influences.

Methods:

Our group originally described the syndrome of Seasonal Affective Disorder (SAD) last year in 29 patients. We recruited a further 50 patients this year and followed them longitudinally through the winter. The clinical profiles of the new cohort were strikingly similar to the initial group. Predominantly women with an onset of their disorder in the twenties, they typically became depressed in the fall and remitted in the spring. Most were bipolar, especially bipolar II with hypomania occurring in spring or summer. About a third of all patients had had no previous treatment. A high percentage of first-degree relatives had a history of affective disorder. Depressions were generally characterized by depressed mood, hypersomnia, hyperphagia, weight gain, carbohydrate craving, low energy level and impaired functioning.

We administered a standardized, structured questionnaire, developed by our group, inquiring into the effects of the changing seasons on mood and behavior, to a group of 40 patients with SAD, 40 patients with bipolar affective disorder from Dr. Elliot Gershon's clinic and 40 age- and sex-matched normal volunteers.

We administered a structured questionnaire developed by Dr. David Rubinow, inquiring into premenstrual mood and behavioral changes in menstruating patients with SAD.

Findings to date:

Approximately 80% of patients had noted that their winter depressions improved when they traveled south. We noted a high correlation between percentage of patients depressed per month (based on history) and photoperiod and monthly temperature for the local area ($r = -0.87$ and -0.98 respectively). There was a significant difference in self-reported seasonal mood variation between SAD patients and bipolars on the one hand, and bipolars and normals on the other. The bipolars showed an intermediate degree of seasonal variability.

Children of patients with SAD show a seasonal variation in birth dates which is ten times greater than that of the general population. This suggests that SAD may be a human equivalent of a seasonal rhythm of reproduction. SAD patients showed greater than normal degrees of depression, hypersomnia and overeating in the premenstrual period compared to a normal population but were not as severely affected in these respects as a group of patients presenting for help specifically because of premenstrual difficulties.

Many of our SAD patients have filled out daily ratings of mood, energy and anxiety and have kept a daily sleep log over many months. An analysis of the patterns of daily self-ratings and their relationship to changing climatic variables may reveal the way in which these changes impinge on mood and behavior.

Significance to Biomedical Research and to the Program of the Institute:

1. The description of SAD has focused attention on a group of patients whose suffering, in many cases, went largely unrecognized. To judge by the number of responses (well over 3,000 from all parts of the country), the problem is not uncommon. Psychiatrists and other physicians should be more aware of the syndrome, especially because it is so easily treated.

2. The careful clinical descriptions we have provided serve as a valuable basis for researchers interested in studying the effect of the climate on mood and behavior. These highly sensitive and reactive individuals are ideal subjects for such studies.

Proposed Course:

In the coming year we propose to extend this study in the following ways:

1. We have observed that many patients who note regular mood changes in conjunction with the seasons have patterns which differ from the typical winter depression with spring-summer hypomania discussed above. Some patients become depressed every spring, others only in fall and others in the summer months. It would be of interest to understand how these subgroups of SAD differ.

2. We are planning to study, together with Drs. Elliot Gershon and John Nurnberger, the pattern and prevalence of seasonally related mood changes in a bipolar population. We intend to investigate whether there are any clinical or family history differences between bipolars with and without seasonal mood changes.

3. In order to evaluate day-to-day changes in mood more efficiently, we have developed daily and weekly self-rating forms, which can be scored by an OpSCAN machine and automatically entered into a computer file. We plan to investigate possible relationships between climatic changes and mood changes in the large group of patients we are following longitudinally.

Publications:

1. Rosenthal, N.E., Lewy, A.J., Wehr, T.A., Kern, H.E., and Goodwin, F.K.: Seasonal cycling in a bipolar patient. *Psychiatry Research* 8:25-31, 1983.
2. Lewy, A.J., Kern, H.E., Rosenthal, N.E., and Wehr, T.A.: Bright artificial light treatment of a manic-depressive patient with a seasonal mood cycle. *Am. J. Psychiatry* 139(11):1496-1498, 1983.
3. Rosenthal, N.E., Sack, D.A. and Wehr, T.A.: Seasonal variation in affective disorders. In *Circadian Rhythms in Psychiatry*, T.A. Wehr and F.K. Goodwin (eds). Boxwood Press, 2-19, 1983.
4. Rosenthal, N.E., Sack, D.A., Gillin, J.C., Lewy, A.J., Goodwin, F.K., Davenport, Y., Mueller, P.S., Newsome, D.A., and Wehr, T.A.: Seasonal affective disorder: a description of the syndrome and preliminary findings with light therapy. *Arch. Gen. Psychiatry* (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center; font-weight: bold;">Z01 MH 02203-01 CP</div>
PERIOD COVERED <div style="font-weight: bold;">October 1, 1982, through September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <div style="font-weight: bold;">Sleep, temperature and activity changes in women with premenstrual syndrome</div>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <div style="font-weight: bold;">Barbara L. Parry, M.D., Medical Staff Fellow, CP, NIMH</div>		
COOPERATING UNITS (if any) <div style="text-align: center;">Biological Psychiatry Branch, NIMH; Clinical Neuropharmacology Branch, NIMH</div>		
LAB/BRANCH <div style="text-align: center; font-weight: bold;">Clinical Psychobiology Branch</div>		
SECTION 		
INSTITUTE AND LOCATION <div style="text-align: center; font-weight: bold;">NIMH, Bethesda, Maryland 20205</div>		
TOTAL MANYEARS: <div style="text-align: center; font-weight: bold;">0.5</div>	PROFESSIONAL: <div style="text-align: center; font-weight: bold;">0.25</div>	OTHER: <div style="text-align: center; font-weight: bold;">0.25</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The symptoms of premenstrual syndrome consist of mood, cognitive, and behavioral disturbances occurring in the premenstrual phase of the cycle. They may become severe enough to cause suicidal depression or psychosis. Objective physiologic parameters that correlate with the subjective symptoms of PMS need to be identified in order to delineate this syndrome further and possibly to suggest better forms of treatment. First, this study will examine <u>sleep, temperature and activity changes across the menstrual cycle</u> in ten women with <u>moderate to severe premenstrual syndrome</u> and in normal volunteers. One month of baseline activity recording, objective ratings and self-ratings of sleep, mood, and energy will be obtained. Subjects will then be admitted to the hospital where they will undergo sleep EEG and temperature recordings two nights a week for the duration of one menstrual cycle.</p> <p>Premenstrual syndrome (PMS) may represent a variant of affective disorder. Therefore, treatment modalities found to be effective in the major affective disorders may be useful in treating patients with PMS. For example, sleep deprivation which induces transient remissions in affective disorder may do the same in PMS. Furthermore, sleep deprivation lowers prolactin, and hyperprolactinemia has been associated with mood disturbances in patients with PMS. Therefore, the effects of sleep deprivation will be investigated in these patients. Prolonged intense light exposure alleviates symptoms in patients with seasonal affective disorder. Since symptoms of SAD and PMS are similar, prolonged intense light exposure will be evaluated as a possible treatment for PMS.</p> <p>Results of sleep deprivation and light treatment experiments may increase our understanding of the pathophysiological mechanisms of PMS and the relationship between PMS and affective disorders.</p> <div style="text-align: center;">(237)</div>		

Other Professional Personnel:

Thomas A. Wehr, M.D.	Acting Chief	CP/NIMH
Wallace Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Norman E. Rosenthal, M.D.	Chief, Outpatient Unit	CP/NIMH
David Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
David Rubinow, M.D.	Staff Psychiatrist	BP/NIMH
Jean Hamilton, M.D.	Medical Staff Fellow	CN/NIMH

Project Description:

A high percentage of women have mood, cognitive and neurovegetative disturbances associated with their menstrual cycle. In particular, in the premenstrual phase they report such symptoms as depression, anxiety, irritability, difficulty concentrating, as well as sleep, appetite and energy disturbances. These symptoms often become severe enough to disrupt normal functioning in work and interpersonal relationships in some, and have resulted in psychosis and suicidal depressions in others. There is some question whether this syndrome may represent a variant of an affective disorder. In order to delineate further premenstrual syndrome (PMS), to understand better its physiologic basis, and to provide potentially better forms of treatment, objective biological variables need to be followed across the menstrual cycle that may then be correlated with subjective mood and behavioral changes. Neuroendocrine parameters have been the focus of previous and ongoing studies. This study will first focus on sleep, temperature and activity changes across the menstrual cycle. Nonpharmacologic strategies for treatment intervention using sleep deprivation or exposure to intense light will be used. Patients with affective disorder frequently show characteristic patterns of change in their sleep, temperature and activity rhythms. Also, sleep deprivation has been found to be effective in ameliorating depressive symptoms in many patients with major affective disorder. By studying these same parameters in patients with premenstrual syndrome, a better understanding of the relationship of PMS to affective disorders may be achieved. Furthermore, sleep and circadian rhythm disturbances appear to play a key role in the pathophysiology of affective disorder. This study will help to determine whether the same is true of PMS.

One major neuroendocrine theory of PMS proposes premenstrual hyperprolactinemia as etiologic in the development of mood symptoms at that time; since sleep deprivation lowers prolactin, sleep depriving women in the premenstrual phase of their cycle may help alleviate symptomatology at that time. In any case, sleep deprivation has antidepressant effects in non-PMS depressives. The time-limited effects of sleep deprivation have restricted its use as a clinical treatment modality in patients with affective disorder. However, since premenstrual syndrome is itself time limited, sleep deprivation, if effective, may be a useful clinical mode of treatment. Anecdotally, women with seasonal affective disorder (SAD) and PMS have reported improvement in their premenstrual symptomatology when treated with light. Therefore, extending the photoperiod by exposure to high intensity light will be tried also in this study (without causing sleep deprivation) to determine its efficacy in relieving symptoms of PMS.

Methods:

Ten women with moderate to severe premenstrual syndrome will be evaluated with screening forms, daily rating forms and personal interviews. Those who meet DSM III criteria for major affective disorder in the premenstrual phase of their cycle only, will be included in the study, as well as age-matched controls. One month of baseline activity recording and self-rating scales will be obtained before admitting the patients to the hospital where sleep and temperature recordings will be obtained two nights per week for the duration of one menstrual cycle. In the third month, sleep deprivation or extended light exposure will be instituted in the premenstrual phase. Blood samples for estrogen, progesterone, and prolactin will be obtained weekly to document ovulation and to correlate behavioral and neuroendocrine events. More conventional modes of treatment for PMS will then be instituted in the event that the aforementioned experimental treatments are not effective.

Findings to date:

The study is currently in its preliminary stages, so there are no findings to date. Sleep and activity changes across the menstrual cycle are suggested by previous reports in the literature.

Significance to Biomedical Research and to the Program of the Institute:

PMS is responsible for morbidity in 40-60% of the female population. No objective physiologic manifestations of PMS have been identified that could be used to follow its course and to measure its response to treatment. Furthermore, current pharmacologic treatment modalities have not been consistently effective and are fraught with side effects. Changes in sleep, temperature and activity across the menstrual cycle may prove to be useful physiological markers of PMS symptoms; also, sleep deprivation or light therapy may be an effective non-pharmacologic treatment. On a conceptual level, the study may elucidate the role of changes in sleep, temperature, activity, and biological clocks in the pathophysiology of PMS.

Proposed Course:

Should total sleep deprivation be an effective clinical treatment of PMS, more specific alterations of sleep schedules such as partial sleep deprivation or shifting the time of sleep will be applied. Should the clinical effects of sleep deprivation be correlated with the lowering of serum prolactin, then a series of pharmacological studies will be done to determine whether or not prolactin inhibition mediates the sleep deprivation response. For example, if nighttime infusions of L-DOPA, which lowers prolactin, are given when subjects are asleep, will this also produce the same antidepressant response as sleep deprivation? Also, if TRH is given acutely, or neuroleptics chronically, to increase prolactin while patients are sleep deprived, will this counteract the antidepressant response of sleep deprivation? Should "light therapy" be effective in ameliorating premenstrual symptoms, a study of women's sensitivity to light across the menstrual cycle will be undertaken to determine whether it might be a state or trait marker.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02205-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of light interventions in seasonal affective disorder (SAD)		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Norman E. Rosenthal, M.D., Staff Psychiatrist, CP, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 0.6	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Having previously shown that extending the photoperiod (day length) with bright artificial light was beneficial to patients with winter depression, we set out to: (1) replicate the finding, (2) examine whether it was mediated by sleep depriving the patients, and (3) investigate how other types of manipulation of environmental light might affect patients with SAD.</p> <p>In six inpatients and seven outpatients we replicated the finding that bright (2500 lux) full-spectrum light has antidepressant effects whereas dim (300 lux or less) light does not. By carefully monitoring wakefulness we established that this effect is not mediated via sleep deprivation (a known antidepressant treatment modality). In an uncontrolled study on eight patients, we showed that bright light in the evening alone had antidepressant effects in six, suggesting that the morning hours are not critical for effective treatment, as may be the case with sleep deprivation.</p> <p>In order to clarify the way in which changes in photoperiod influence mood, we exposed two SAD patients to an artificially shortened day length and later to dim environmental light for two one-week periods in an isolated environment during the spring months when they were euthymic. Neither of these conditions induced depression in either patient, contrary to our predictions. It is possible that SAD patients may be refractory to photoperiodic manipulations at certain times of the year, a phenomenon seen in certain animals.</p> <p>Further studies to elucidate the mechanisms involved in causing and reversing winter depressions are being planned. These include further photoperiodic manipulations, as well as the administration to patients of the hormone melatonin, which serves as a chemical transducer of darkness in animals.</p>		
(241)		

Other Professional Personnel:

David A. Sack, M.D.	Chief, Clinical Research Unit	CPB/NIMH
Steven P. James, M.D.	Clinical Associate	CPB/NIMH
Barbara L. Parry, M.D.	Clinical Associate	CPB/NIMH
Wallace B. Mendelson, M.D.	Chief, Unit on Sleep Studies	CPB/NIMH
Thomas A. Wehr, M.D.	Acting Chief	CPB/NIMH

Project Description:

The close correlation between the timing of symptoms in SAD and the variation in climatic variables, most notably day length and temperature, led us to hypothesize that one or more of these variables are responsible for inducing, maintaining and reversing the depression. By manipulating such variables, one might be able to change the pattern of symptoms, thus clarifying the mechanism of this relationship and perhaps treating the symptoms.

Methods:

In our initial study last year we showed that by extending the photoperiod with bright (2500 lux) full-spectrum light for three hours before dawn and three hours after dusk during the winter days we were able to reverse the depressive symptoms within three or four days. Dim (100 lux) yellow light used as a control in the same way had no consistent effect.

One central problem of this study is that wakefulness during treatments was not systematically ensured, leaving open the possibility that the active principle was sleep deprivation and that bright light was simply a more effective sleep depriver than dim light. This year we replicated our initial study, extending the photoperiod with bright (2500 lux) and dim (300 lux) full-spectrum light in six inpatients and seven outpatients. Wakefulness was ensured in the inpatients by nurses' checks and sleep recordings and in the outpatients by telephone calls and self-ratings.

In addition to this, we treated eight patients in an uncontrolled pilot study with bright light in the evening alone to test whether the morning hours with their attendant sleep deprivation were critical to the light response.

In order to further understand the mechanism of the effect of light on mood, we attempted to induce depression in two highly light-responsive SAD patients this spring after they had recovered from their winter depressions. We studied each patient in a room isolated from all natural fluctuations in light and exposed each to a short skeleton photoperiod, i.e., a nine-hour period during which the subject is exposed to very bright light for the first and last three hours and very dim light for the middle three hours.

Findings to date:

1. We found that extending the photoperiod with bright full-spectrum light produced significant antidepressant effects in both inpatients and outpatients. Relapse occurred to a significant degree within a few days of stopping the light treatment. We were thus able to replicate our findings from the previous winter.

2. Very little sleep occurred during light treatment under both treatment conditions and there was no difference in sleep length between the two conditions. We therefore conclude that the antidepressant effects of light are not mediated via sleep deprivation.

3. Six out of eight subjects treated in an uncontrolled way with evening light only showed a clean-cut antidepressant response. This result suggests that the morning hours are not critical for the antidepressant effects of light treatment. This finding should improve design of future studies because treatment in the evening only avoids the confounding variable of sleep deprivation.

4. We had predicted that our two SAD patients would become depressed during the week on a short skeleton photoperiod. This did not occur in either case. We then exposed both subjects to a week of dim days (16 hours of low intensity light) and again neither patient became depressed. We think that the most likely explanation for this is that mood in SAD patients is relatively refractory to change as a result of light manipulations in the spring.

Significance to Biomedical Research and to the Program of the Institute:

1. The introduction of light as an antidepressant treatment promises relief to the large numbers who suffer from SAD, a condition for which light appears to be both an effective and a safe treatment. The therapeutic effects of light may extend to other conditions, e.g., non-seasonal affective disorder.

2. Light promises to be a valuable research tool for understanding SAD and perhaps affective disorders in general. It is easily manipulated and appears to have powerful, predictable and replicable effects in winter depression. By studying these effects and their physiological correlates, we hope to learn more about these conditions.

3. Patients with SAD may represent the extreme in the spectrum of human vulnerability to seasonal changes. Many people who would not meet the criteria for SAD may well be influenced by climatic changes, resulting in human suffering and decreased productivity. Environmental light manipulations might improve the quality of life for such people.

4. Light treatment may be beneficial in conditions other than SAD. Non-seasonal depression and delayed phase sleep syndrome are two likely possibilities. Our demonstration of the therapeutic efficacy of light may encourage others to explore its use in different clinical settings.

Proposed Course:

1. We plan to continue our skeleton photoperiod studies to determine whether the length of light rather than simply the amount of light is important in mediating its effects. Results of these experiments may establish a link to photoperiod controlled seasonal behaviors in animals, where underlying mechanisms have already been elucidated.

2. We plan to explore the most efficient way of delivering light, e.g., the minimum time and frequency of exposure necessary for an antidepressant effect.

3. We intend to explore which other patient groups might benefit from light treatment, e.g., non-seasonal depressions and patients with delayed phase sleep syndrome. We also plan to investigate the effects of increasing exposure to bright light in winter in normal subjects.

4. The pineal gland is almost always involved in light-induced seasonal changes in animals. We shall try to uncover the mechanism of light treatment by attempting to block the light effect, e.g., by the administration of the pineal hormone, melatonin, or other pharmacological agents.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02206-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of seasonal affective disorder (SAD)		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Norman E. Rosenthal, M.D., Staff Psychiatrist, CP, NIMH		
COOPERATING UNITS (if any) Laboratory of Clinical Studies, NIAAA		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.4	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>There is a well established epidemiological association between the incidence of affective episodes and suicides and the seasons. Our group has previously described patients who regularly become depressed each winter, and we have called this syndrome Seasonal Affective Disorder (SAD). One approach to understanding how changes in mood are mediated by seasonal changes in this population is to study physiological and biochemical parameters at different times of the year in people whose mood is vulnerable to seasonal changes and in healthy controls. This may yield insights into the mechanism of seasonal influences on the incidence of affective episodes in the general population.</p> <p>We have shown that patients with SAD (nine studied so far) have significantly increased sleep length and sleep latency (the time it takes to fall asleep) but a significant decrease in slow wave (delta) sleep in the winter. These seasonal changes have not been found in healthy volunteers.</p> <p>Patients with SAD complain of hypoglycemia symptoms in the winter only. We are currently performing glucose tolerance tests on patients and controls in winter and summer. A marked seasonal variation in brain serotonin and platelet serotonin reuptake has been described. We collected 24-hour urine specimens and, in collaboration with Dr. Markku Linnoila, have measured serotonin and the serotonin metabolite, 5HIAA, in urine in summer and winter in eight subjects but have found no seasonal differences in these variables thus far. Future plans involve a more extensive biological work-up of patients and controls.</p>		
(245)		

Other Professional Personnel:

David A. Sack, M.D.	Chief, Clinical Research Unit	CP/NIMH
Steven P. James, M.D.	Clinical Associate	CP/NIMH
Barbara L. Parry, M.D.	Clinical Associate	CP/NIMH
Wallace B. Mendelson, M.D.	Chief, Unit on Sleep Studies	CP/NIMH
Thomas A. Wehr, M.D.	Acting Chief	CP/NIMH
Markku Linnoila, M.D., Ph.D.	Clinical Director	LCS/NIAAA

Project Description:

There is a well-established epidemiological association between affective episodes, suicides and the seasons.

The introduction of light as an antidepressant treatment promises relief to the large numbers who suffer from Seasonal Affective Disorder (SAD), a condition for which light appears to be both effective and safe. The therapeutic effects of light may extend to other conditions, e.g., non-seasonal affective disorder.

We have attempted to understand how the changing seasons might alter mood by studying physiological variables at different times of the year.

Selection of Subjects:

SAD patients were screened and selected on the basis of the following criteria:

- (a) met RDC criteria for major affective disorder at some time in their lives;
- (b) depressions occurred during at least two successive winters, and remitted by the following summers;
- (c) there were no obvious seasonally fluctuating psychosocial causes to account for the seasonal occurrence of depressions.

Methods:

Nine subjects with SAD and eight normal controls had EEG recordings during sleep for one adaptation night and three subsequent nights both summer and winter.

During their admission 24-hour urine collections were obtained from patients.

Findings to date:

In our 1981-1982 study we showed seasonal sleep changes in SAD patients (increased sleep latency, increased sleep length and a marked decrease in delta sleep in the winter). This year we showed that these changes were not present in eight normal volunteers studied in summer and winter. We also repeated summer and winter sleep studies in ten other SAD patients; these data await analysis.

The reduction in delta sleep was marked (approximately 50%) and has been described in other depressed populations. Such a reduction has been ascribed to a reduced level of some as yet undefined sleep factor by some theorists. This may account for why patients' sleep is shallow and why, despite their increased sleep length, they do not feel refreshed during the day. Growth hormone (GH) secretion has been associated with slow wave sleep, and it would be of interest to measure this in view of the reduction in slow wave sleep.

Several patients with SAD complain of symptoms which suggest hypoglycemia in winter only. This may reflect a seasonal variation in carbohydrate metabolism. One subject was given a glucose tolerance test (GTT) last winter and showed a typical hypoglycemia response.

A marked seasonal variation in brain serotonin and platelet serotonin uptake has been described. We collected 24-hour urine samples from eight SAD patients last summer and winter and, in collaboration with Dr. Markku Linnoila, have measured serotonin and the serotonin metabolite, 5HIAA. Thus far we have found no differences.

Significance to Biomedical Research and to the Program of the Institute:

1. A large number of people appear to be adversely affected by changes in seasons and the weather. It would be valuable to learn how these changes are mediated. This might contribute to our ability to protect people from these adverse effects.

2. Since seasonal variation appears to be a cardinal feature of the affective disorders, an understanding of the pathophysiology of these changes might expand our understanding of the mechanisms underlying the affective disorders.

Proposed Course:

1. We plan to follow up on our sleep findings by analyzing about 140 sleep records collected from normals and SAD patients at different times of the year.

2. We intend to collect summer and winter 24-hour plasma profiles of a variety of hormones including melatonin, prolactin, growth hormone and cortisol, all of which have been implicated in the changes seen either in depression, or with the changing seasons or in both situations. We are also planning to perform glucose tolerance tests in summer and winter on SAD patients and normal volunteers.

3. We plan to study neuroendocrine and sleep changes resulting from interventions with light to be undertaken this coming winter.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01850-06 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical pharmacology of antidepressants		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) William Z. Potter, M.D., Ph.D., Clinical Psychobiology Branch, NIMH		
COOPERATING UNITS (if any) Pharmacology-Toxicology Program, NIGMS; Laboratory of Clinical Science, NIMH; Division of Special Mental Health Research, NIMH; Cl. Neuropharma. Br., NIMH; Laboratory of Psychol. and Psychopathol., NIMH; Biol. Psychiat. Br., NIMH		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.5	PROFESSIONAL: 4.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Antidepressant medications are prescribed to millions of Americans, many of whom receive the drug for months to years. Despite increasingly sophisticated studies in animals and the development of more <u>biochemically specific antidepressants</u>, the therapeutic mechanism of action in man remains unknown. <u>Comparison of biochemical effects in CSF, plasma and urine in the same patients is now feasible with new, efficient high performance liquid chromatography assays</u>, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. Findings of particular interest include the following:</p> <ol style="list-style-type: none"> 1. Effects on <u>norepinephrine (NE)</u> of several antidepressants instead of on serotonin (5HT) or dopamine (DA) support a hypothesis that a prerequisite for therapeutic action is <u>increased efficiency</u> of the noradrenergic system associated with stabilized regulation of other systems dependent on NE. 2. <u>Differential responses of NE to lithium but not zimelidine</u>, a 5HT, or desipramine, a NE uptake inhibitor, occur in healthy volunteers vs. patients. This provides a new lead on the bidirectional effect of lithium. 3. Reversal of certain specific alcohol-induced memory deficits by zimelidine is an exciting finding consistent with our previous finding that it stimulates the peptide, vasopressin, which is implicated in memory function. 4. Chinese vs. caucasian differences in dose requirements is explained by differences in <u>drug metabolism</u> although the previously hypothesized responsible enzymatic step has been ruled out. Ours is the first data challenging a generally held assumption about relevant <u>pharmacogenetics</u>. 5. Clorgyline, a specific monoamine oxidase Type A inhibitor, has now been found to show potent antidepressant effects in unipolar as well as bipolar patients who have failed to respond to other treatments, including ECT. 		

Other Professional Personnel:

Markku Linnoila	Staff Psychiatrist	CP/NIMH
Richard Ross	Staff Fellow	CP/NIMH
Matthew Rudorfer	Staff Fellow	P-T/NIGMS
Thomas Wehr	Chief, Clinical Research Unit	CP/NIMH
Mika Scheinin	Visiting Fellow	CP/NIMH
Mingdao Zhang	Visiting Fellow	CP/NIMH
Markku Koulu	Guest Worker	CP/NIMH
Timo Seppala	Visiting Associate	CP/NIMH
Elizabeth Lane	Visiting Fellow	P-T/NIGMS
Anthony Zavadil	Guest Worker	LCS/NIMH
Irwin J. Kopin	Chief	LCS/NIMH
Richard J. Wyatt	Chief	DSMHR/NIMH
Dennis L. Murphy	Chief	CN/NIMH
Farouk Karoum	Research Chemist	DSMHR/NIMH
John Nurnberger	Staff Psychiatrist	BPB/NIMH
Elliot Gershon	Chief, Section on Psychogenetics	BPB/NIMH
Herbert Weingartner	Psychologist	LPP/NIMH

Project Description:

The major thrust is to understand the effects of major somatic antidepressant treatments on the monoamine neurotransmitter systems in man. Systematic studies of drug action in normal volunteer controls and depressed patients controlling for pharmacokinetic and pre-drug physiologic variance has permitted demonstration of both predicted and unexpected biochemical alterations following norepinephrine (NE) and serotonin (5HT) uptake inhibition, monoamine-oxidase Type A inhibition, lithium and electro-convulsive therapy (ECT). These treatments share the pharmacodynamic action of reducing NE turnover while increasing NE function.

Comparison of biochemical effects in CSF, plasma and urine in the same patients is now feasible with new, efficient high performance liquid chromatography assays, and, when coupled with physiologic, behavioral and neuroendocrine measures, allows for clearer systems interpretations of changes. State-of-the-art measures of NE, 5HT, DA and their metabolites are made under controlled conditions both cross sectionally in time and longitudinally in order to identify interrelationships, to test assumptions about the regulation of these neurotransmitter systems, and therefore to definitively describe effects of antidepressants as they relate to these neurotransmitter systems.

Methods:

The neurotransmitter systems of patients with either unipolar or bipolar major affective disorder are characterized after at least a 3-week drug-free period and then between the 3rd and 5th week following antidepressant treatment. Certain parameters, such as urinary transmitter and metabolite concentrations, are studied repeatedly following the beginning of each treatment. Parallel studies are performed in healthy volunteers when feasible as described below.

Treatments are ideally administered so as to produce maximal effects on the presumed target biochemical system such as inhibition of NE uptake after desipramine (DMI), of 5HT uptake after zimelidine (ZIM), and of MAO-Type A after clorgyline using control of pharmacokinetic variance (blood levels of DMI and ZIM) or biochemical indices (MHPG decrease after clorgyline). In the case of lithium and ECT, standard regimens are followed.

Studies in college age volunteers housed on the unit are of shorter duration (up to two weeks of active drug) and include DMI, ZIM and lithium. In addition, interactions of these drugs with alcohol on behavioral and physiologic parameters are measured in volunteers but not depressed patients.

Specialized pharmacokinetic and baseline biochemical studies are performed in volunteers age- and sex-matched to our accumulated patient population. These volunteers come to the clinic on the day of the study.

New, improved and efficient high performance liquid chromatography assays are being developed to study NE, 5HT and DA. These are based on the synthesis of appropriate internal standards, utilization of advances in electrochemical detection, and application of automated techniques to the more "routine" analyses. GC-MS has continued to be used for multiple measures in urine.

Findings to date:

1. Effects on norepinephrine (NE) of several antidepressants distinct from those on serotonin (5HT) or dopamine (DA) support a hypothesis that a prerequisite for therapeutic action is increased efficiency of the noradrenergic system which is associated with stabilized regulation of other systems dependent on NE. Decreased turnover is primarily shown by reduction of the sum of NE and metabolites in urine although MHPG in plasma and CSF demonstrate the same effect but not as quantitatively. The proportional increase of NE in plasma following an orthostatic challenge (lying to standing) combined with cardiovascular parameters is the basis for concluding that there is increased efficiency.

2. Lithium treatment is associated with a marked reduction in NE turnover in middle age patients but not in young healthy volunteers, whereas zimelidine and desipramine produce similar changes in the two groups. This provides our first evidence that the biochemical effects of lithium may be dependent on some abnormality of baseline biochemical function and thus separate it from two other classes of antidepressant agents. Other evidence suggests that changes after MAOI's may be similar in volunteers and patients. The dependency of lithium's effects on state suggests a clue to its bidirectional actions.

3. Reversal of certain specific alcohol-induced memory deficits by zimelidine is an exciting finding consistent with our previous finding that it stimulates the active peptide, vasopressin, which is implicated in memory functioning. It is also of interest that zimelidine reduces drinking behavior in man and that other putative indirect 5HT agonists reduce ethanol consumption in rodents.

4. We had expected that differences between Chinese and Caucasians in the metabolism of tricyclic antidepressants which we previously reported were based

on differences in hydroxylation. Using a urinary "metabolic ratio" of debrisoquine and its metabolites--a proposed model for drug hydroxylation--we found no relationship between slow metabolism of debrisoquine and of DMI. More particularly, there was no relationship between the specific hydroxylation clearance of DMI and the apparent hydroxylation of debrisoquine. These findings are not consistent with predictions made from *in vitro* experiments using rat liver microsomes. The debrisoquine metabolic ratio is already being used worldwide as a tool to investigate pharmacogenetic population differences, although it has never been validated. Our findings directly challenge the validity of this test and emphasize the necessity of detailed metabolic studies to understand pharmacokinetic variance of specific compounds.

5. Clorgyline, in doses of 5-15 mg/day (one third of those originally used), has been found to be a potent antidepressant in unipolar as well as bipolar patients.

Significance to Biomedical Research and to the Program of the Institute:

Understanding of the mechanism(s) of action of antidepressant treatments produces improved therapeutics, new drugs, tools for studying and investigating the underlying pathophysiology of depression and therefore, ultimately, provides the basis for prevention.

From a therapeutic point of view pharmacokinetic studies have been critical to removing problems related to inappropriate dosing. Moreover, the systematic study of biochemically selective (clorgyline) and frequently less toxic agents (zimelidine) provides treatments which are effective in many patients who do not respond to standard antidepressants.

Of ultimate importance is the continued finding that changes of the noradrenergic system are always involved in the process of somatic antidepressant treatments. Although simple deficit or excess catecholamine hypotheses of depression do not explain drug action, it seems clear that to understand the mechanism we must understand the role of NE. More and more investigators are "returning" to studies of the NE system in man.

Proposed Course:

1. Complementary studies with newly available selective NE and 5HT uptake inhibitors are planned to test the generalizability of our findings. A collaborative study on the biochemical effects of ECT will be undertaken to replicate and extend the results found in a small number of inpatients here.
2. Detailed investigation of factors controlling the elimination of NE and metabolites from plasma will be performed in volunteers so as to understand the apparent dissociation of results from those seen in urine.
3. Selected chronic volunteer studies will be performed in subjects age- and sex-matched to patients to see if differential drug effects are really related to pathophysiology of depression.
4. Alcohol interaction studies will only be done in collaboration with the new intramural NIAAA program.

5. Intravenous acute challenge tests using selective NE and 5HT uptake inhibitors will be developed to study the relationship between these two systems and drug response.

6. Pharmacokinetic studies will be limited to control of variance unless new cross-cultural populations become available.

Publications:

Potter, W.Z. and Linnoila, M.: Tricyclic antidepressant concentrations: Clinical and research implications. In Neurobiology of the Mood Disorders, R.M. Post and J.C. Ballenger (eds.). William and Wilkins, Baltimore, Maryland, 698-709, 1983.

Linnoila, M., Karoum, F., Calil, H.M., Kopin, J. and Potter, W.Z.: Alteration of norepinephrine metabolism with desipramine and zimelidine in depressed patients. Arch. Gen. Psychiatry 39:1025-1028, 1982.

Linnoila, M., Lamberg, B.-A., Potter, W.Z., Gold, P.W. and Goodwin, F.K.: High reverse T_3 levels in manic and unipolar depressed women. Psych. Res. 6: 271-276, 1982.

Potter, W.Z.: Clinical pharmacokinetics of antidepressants. In Guidelines for the Use of Psychotropic Drugs, H. Stancer et al. (eds.). Spectrum Publications, Inc., 119-137, 1983.

Linnoila, M., Insel, T., Kilts, C., Potter, W.Z. and Murphy, D.L.: Plasma steady-state concentrations of hydroxylated metabolites of clomipramine. Clin. Pharm. Ther. 33:429-437, 1983.

Potter, W.Z., Rudorfer, M.V. and Lane, E.A.: Active metabolites of antidepressants: pharmacodynamics and relevant pharmacokinetics. In Frontiers in Biochemical and Pharmacological Research in Depression, E. Usdin (ed.), Raven Press, New York, in press.

Potter, W.Z., Lane, E.A. and Rudorfer, M.V.: Hydroxy-metabolite concentrations: role of renal clearance. In Clinical Pharmacology in Psychiatry: (Third International Meeting), E. Usdin, S. Dahl and L.F. Gram (eds.). Macmillan Press, Ltd., Basingstoke, England, in press.

Linnoila, M., Karoum, F., Potter, W.Z.: Effects of antidepressant treatments on "whole body" norepinephrine turnover. In Clinical Pharmacology in Psychiatry, (Third International Meeting), E. Usdin, S. Dahl and L.F. Gram (eds.), Macmillan Press, Ltd., Basingstoke, England, in press.

Ross, R.J., Zavadil, A.P., Calil, H.M., Linnoila, M., Kitanaka, I., Blomberg P., Kopin, I.J. and Potter, W.Z.: Effects of desmethylinipramine on plasma norepinephrine, pulse, and blood pressure. Clin. Pharmacol. Ther. 33:429-437, 1983.

Linnoila, M., Karoum, F. and Potter, W.Z.: Effects of antidepressant treatments on dopamine turnover in depressed patients. Arch. Gen. Psychiatry, in press.

Scheinin, M., Chang, W.-H., Kirk, K. and Linnoila, M.: Simultaneous determination of 3-methoxy-4-hydroxyphenylglycol, 5-hydroxyindoleacetic acid and homovanillic acid in cerebrospinal fluid with high performance liquid chromatography using electrochemical detection. Anal. Biochem. 131:246-253, 1983.

Garrick, N.A., Scheinin, M., Chang, W.-H., Linnoila, M. and Murphy, D.L.: Differential effects of clorgyline on catecholamine and indoleamine metabolites in the cerebrospinal fluid of rhesus monkeys. Biochem. Pharmacol., in press.

Weingartner, H., Rudorfer, M.V., Buchsbaum, M.S. and Linnoila, M.: Effects of serotonin on memory impairments produced by ethanol. Science, in press.

Potter, W.Z., Linnoila, M., Karoum, F., Wyatt, R.J. and Goodwin, F.K.: Common mechanism of action of biochemically "specific" antidepressants. In Prog. Neuro-psychopharmacol. and Biol. Psychiat. 7:311-316, 1983.

Mellstrom, B., Alvan, G., Bertilsson, L., Potter, W.Z., Sawe, J. and Sjoqvist, F.: Nortriptyline formation after single oral and intramuscular doses of amitriptyline. Clin. Pharm. Ther. 32:664-667, 1982.

Potter, W.Z.: Rapid cycling disorder: A psychopharmacologic approach. In Affective Disorders Reassessed: 1983, F.J. Ayd (ed.), Ayd Medical Communications, Baltimore, Maryland, in press.

Ross, R.J., Waszczak, B.L., Lee, E.K. and Walters, J.R. Effects of benzodiazepines on single unit activity in the substantia nigra pars reticulata. Life Sci. 31:1025-1035, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00449-09 CP
PERIOD COVERED October 2, 1982, through May 16, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Outpatient followup studies of manic-depressive patients and families		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Yolande Davenport, Chief, Unit on Family Studies, CPB, NIMH		
COOPERATING UNITS (if any) <div style="text-align: center;">Laboratory of Developmental Psychology and Biological Psychiatry Branch, NIMH</div>		
LAB/BRANCH <div style="text-align: center;">Clinical Psychobiology Branch</div>		
SECTION		
INSTITUTE AND LOCATION <div style="text-align: center;">NIMH, Bethesda, Maryland 20205</div>		
TOTAL MANYEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">0.75</div>	OTHER: <div style="text-align: center;">0.75</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Manic depressive illness is a familial disorder with a generally acknowledged biogenetic component. Through studies of bipolar patients and their families, it is possible to learn more about the manner in which genetic predisposition may interact with psychocultural mechanisms of transmission to precipitate the disorder. The research efforts of the Unit on Family Studies were focused in the following areas: <u>collaborative studies of the cognitive and emotional development of children born to families where one parent has bipolar disorder</u>; <u>studies of early nurturing patterns of parents of infants at risk</u>; <u>follow-up and outcome studies of rapid-cycling bipolar patients</u>; <u>studies of psychosocial factors associated with seasonal affective disorders</u>; <u>a study of themes and therapeutic factors found in two groups of symptomatically distinct married bipolar patients</u>; and <u>a study of rapid cycling patients who may have a vulnerability to the development of multiple sclerosis</u>.</p> <p>A study of the nurturing attitudes and practices of a group of bipolar patients and their infant children confirmed hypotheses reported in earlier studies regarding the influence of the early rearing environment of the potential manic depressive patient. A study of rapid cycling patients who may be vulnerable to multiple sclerosis continues with a focus on examining family pedigrees in order to assess the absence or presence of neurological disorders. A preliminary case report is in press. Follow-up studies of rapid cycling and seasonal disorder bipolar patients are continuing.</p>		

Other Professional Personnel:

Thomas A. Wehr	Acting Chief	CP/NIMH
Marvin L. Adland	Consultant	CP/NIMH
H. Arnold Meyersburg	Consultant	CP/NIMH
David Sack	Staff Psychiatrist	CP/NIMH
Leon Cytryn	Medical Officer (Research)	LDP/NIMH
Donald McKnew	Medical Officer (Research)	LDP/NIMH
Martine Lamour	Medical Officer (Research)	LDP/NIMH
Marion Yarrow	Chief, Lab. of Dev. Psych.	LDP/NIMH
Carolyn Waxler	Research Psychologist	LDP/NIMH
Norman Rosenthal	Staff Psychiatrist	CP/NIMH
Richard Ross	Senior Staff Fellow	CP/NIMH
Charles Kellner	Medical Staff Fellow	BP/NIMH
Robert Post	Chief	BP/NIMH
Laurent Holt	Social Work Intern	CP/NIMH
Kathe Schwartzberg	Social Work Intern	CP/NIMH
Ellen Joyce	Social Work Intern	CP/NIMH
Lisa Coleman	Social Work Intern	CP/NIMH

Project Description:

Our collaborative research with the Laboratory of Developmental Psychology involving children born to parents treated in our psychotherapy groups continued this year. Study results indicate that, when compared to matched controls from normal families, by age two children with a bipolar parent were already found to be experiencing significant psychiatric problems. The ways in which early problems become embedded in social relationships with peers was examined. It was found that children from bipolar families had difficulty in handling hostility, in sharing, and generally showed more maladaptive patterns of aggression. These impairments were strikingly similar to the recurring interpersonal problems attributed to their manic-depressive parents in our earlier reports.

When nurturing attitudes and behaviors of bipolar parents were compared with parents without psychopathology, differences were also found based on the Block Q Sort ratings. Mothers from bipolar families were found to be less attentive to their child's health needs, emphasized performance in achievement-related areas, were less likely to value openness to new experiences for their child (were more overprotective), and reported more negative affect toward the child. Home visitors observed bipolar mothers to be more disorganized, less active in interaction with their child, and more unhappy, tense, and ineffective. On a global functioning scale, bipolar parents secured lower scores in the areas of family interaction and social adjustment. Situational problems of considerable severity were experienced by all bipolar families, including the occurrence of clinical depression in the well parent. Four papers resulting from these studies were accepted this year as part of a special section for publication in the American Journal of Psychiatry.

A study to examine history and outcome of patients previously hospitalized on the Inpatient Unit with a rapid-cycling mood disorder continues. The focus this year has resulted upon validating the selection of various mood rating

instruments suitable for telephone use with these patients. Although the course of rapid-cycling patients is precarious because of their refractoriness to treatment, preliminary data indicates that they tend to follow a highly variable course after discharge. Interim history since discharge, the incidence and intensity of recurring mood episodes, incidence of relapse and rehospitalization, the use of other treatment interventions since discharge, and the overall level of functioning achieved at the time of follow-up will be evaluated in the study.

A study of married rapid-cycling bipolar patients and their spouses who were seen over a nine-month period in a couples' psychotherapy group also continues. The index patients, previously hospitalized in the Inpatient Unit, presented stormy histories of rapidly alternating manic and depressive episodes, each phase lasting two to six weeks. On admission to the group, the rapid-cycling patients were relatively stable on medication. However, marital problems, some of which were necessarily dormant during earlier periods of coping with the illness and rearing children, surfaced and were brought to the psychotherapy group for discussion. The purpose of the study was to observe whether specific differences existed in the defensive techniques of families with a rapid-cycling member in contrast to bipolar families previously studied where episodes occurred less frequently, less predictably, and with long periods of euthymia between episodes. The following major patterns and themes have emerged: families may develop a phenomenon we have termed "family cycling" in response to the cycling mood of the index patient; role diffusion is pronounced; fear of recurrence of the illness is more pervasive and anxiety-producing in rapid-cycling families and the thematic degree of denial of affect, dependency, and loss is of the same magnitude found in other bipolar families. Although rapid-cycling illness is not rare, there is little in the literature describing the experience of these patients and their families, and the adjustments they make to the illness. The completed study will include a discussion of psychotherapeutic treatment of these families. A paper is near completion.

A study of familial influence was begun this year as the consequence of finding multiple sclerosis in several diagnosed manic-depressive inpatients. It is proposed to reexamine a large group of bipolar patients, and their relatives if possible, using a structured interview form designed to ascertain the presence of previously reported neurologically based disorders in families. A case report of preliminary findings and recommendations is in press.

The Unit remained highly involved in the Seasonal Affective Disorder studies, providing psychosocial assessments of all patients admitted to the Clinic, psychotherapeutic support on a sustained basis as needed, and participating in blind ratings of patients. Individual, couples', and family psychotherapy was available to these patients. At termination, referral to community resources was provided when indicated.

The Unit on Family Studies also continued to provide a research training experience for graduate social work students, sponsoring and supervising their placement in the Branch. The students were assigned to both Inpatient Unit and Outpatient Clinic for this clinical experience. As a cooperative

effort, CPB investigators and the Chief of the Outpatient Unit participated with the Family Studies Unit in providing a training program in psychological research. Students participated in a carefully supervised program of clinical assessment, psychotherapy and research. The training program was designed to illustrate the synthesis of the approaches of different disciplines, the ways in which clinical and biological research can synergize with rather than detract from clinical care, and an appreciation of the complex interactions between psychosocial factors and biologic predispositions. The program has been highly praised by the collaborating schools of social work.

Significance to Biomedical Research and to the Program of the Institute:

It is widely agreed that manic-depressive illness is familial. The Unit has therefore sought to provide clinical care to patients and their families, while continuing to examine the impact of psychocultural and psychodynamic features on a disorder assumed to have a strong biological component. Through examination of the dysfunctional interactional patterns appearing in these families, evaluations have been made regarding efficacious medical and psychotherapeutic treatment approaches.

Proposed Course:

The long term research of the Unit remains focused on better understanding of the interplay between biogenetic factors and environmental events. The direction of the Unit this year will continue concentration on intensive study of subgroups of patients with affective disorders and their families.

Publications:

Davenport, Y.B., Zahn-Waxler, C., Adland, M.L., and Mayfield, A. Early child rearing practices in bipolar families. In press. Am. J. Psychiatry.

Zahn-Waxler, C., McKnew, D.H., Cummings, E.M., Davenport, Y.B., and Radke-Yarrow, M. Problem behaviors and peer interactions of young children with a manic-depressive parent. In press. Am. J. Psychiatry.

Kellner, C.H., Davenport, Y.B., Post, R.M., Ross, R.S. Rapid-cycling bipolar disorder and multiple sclerosis: two case reports. In press. Am. J. Psychiatry.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02192-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Sleep in psychiatric and endocrine disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Wallace B. Mendelson, M.D., Chief, Unit on Sleep Studies		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH Child and Family Research, NICHD Clinical Neuropharmacology Branch, NIMH Neonatal and Pediatric Medicine Branch, NICHD		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Unit on Sleep Studies		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 2.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> These studies represent collaborative projects with investigators interested in related issues which bear on sleep physiology. Included are a project showing decreased REM latency in <u>panic-anxiety disorder</u> patients, complementing a previous study here which showed the same process in obsessive-compulsive disorder patients. Following a long-term interest of the laboratory on <u>sleep-related growth hormone secretion</u>, patients with short stature and growth hormone neurosecretory dysfunction were found to have decreased number and amplitude of growth hormone pulses around the 24 hours, suggesting that there is a spectrum of GH regulatory dysfunction from absolute deficiency to irregularity of secretion. A variety of <u>circadian rhythm</u> studies continue, with evaluation of the sleep-waking aspects of <u>longitudinal</u> studies in patients with bipolar depressive illness and <u>seasonal depression</u>. In the latter group, seasonal variations in amounts of slow-wave sleep have been found. </p>		

Other Professional Personnel:

Thomas Uhde, M.D.	Chief, Unit on Anxiety and Affective Disorders, BPB, NIMH
William Sonis, M.D.	Medical Staff Fellow, Child and Family Research, NICHD
David Sack, M.D.	Chief, Clinical Research Unit, CP, NIMH
Thomas Wehr, M.D.	Acting Chief, CP, NIMH
Norman Rosenthal, M.D.	Chief, Outpatient Unit, CP, NIMH
Bessie Spiliotis, M.D.	Medical Staff Fellow, Neonatal and Pediatric Medicine Branch, NICHD
Judith Rapoport, M.D.	Chief, Section on Childhood Mental Illness, Biological Psychiatry Branch, NIMH
Thomas Insel, M.D.	Clinical Associate, Clinical Neuropharmacology Branch, NIMH
Robert Post, M.D.	Chief, Biological Psychiatry Branch, NIMH

Project Description:

1) Sleep in panic-anxiety syndromes (with Dr. Thomas Uhde) and other disorders. Patients with panic-anxiety like obsessive-compulsive patients, are often helped by antidepressants, raising the possibility that some aspects of their physiology might have traits in common with depression. For this reason, and because their sleep has never been well characterized, sleep studies were performed in nine patients. Other disorders which continue to be studied include obsessive-compulsive children (with Dr. Judith Rapoport) and adult depressives treated with carbamazepine (with Dr. Robert Post). A study of the Genain quadruplets, concordant for schizophrenia, showed great variability in sleep between individuals.

2) Sleep and growth hormone secretion in children with growth hormone neurosecretory dysfunction (with Dr. William Sonis). The Unit has a long history of characterizing the mechanism of secretion of growth hormone (GH). In normals, 80% of the typical 24-hour secretion occurs in one major episode about 90-minutes after sleep onset (Mendelson, 1982). As a further step in this series of projects, we recorded sleep from a series of patients with neurosecretory dysfunction.

3) Support service for circadian rhythms studies (with Drs. Thomas Wehr, David Sack and Norman Rosenthal). The Clinical Psychobiology Branch (CPB) engages in a variety of studies of circadian rhythms and psychiatric disorders, of which sleep studies are an integral part.

These include sleep monitoring of patients in temporal isolation, seasonal changes in sleep in normal volunteers and patients with seasonal depressions, monitoring of state of consciousness in seasonal depressives undergoing light treatments, monitoring of depressed patients in partial sleep deprivation, and longitudinal studies of bipolar patients.

Methods:

All studies involve standard techniques of sleep recording, which involve electrode placements for electroencephalogram, electro-oculogram and electro-

myogram. Recordings are performed on Grass Model 7 polygrapher calibrated to 50 microvolt/7.5 mm with a paper speed of 10 mm/rec. In any one study, records are read by one technician who interprets a sleep stage for each 30 second epoch.

Findings to Date:

- 1) The major finding of this project is that panic-anxiety patients have a decreased REM latency compared to laboratory norms.
- 2) These patients are children with normal response to provocative growth hormone secretion tests, but low somatomedin-C levels and abnormal secretory patterns. It was found that these children had decreased number and amplitude of GH secretory pulses for 24-hours.
- 3) Findings in collaborative circadian rhythm studies are found in the appropriate parts of the CPB Annual Report. Among the most striking observation have been a decreased slow-wave sleep during the winter in patients with seasonal depression.

Significance to Biomedical Research and to the Program of the Institute:

- 1) Originally, short REM latency was thought to be a sleep finding characteristic of depression. Further work from this Unit, in collaboration with Dr. Tom Insel, showed that adult patients with obsessive compulsive disorder (which is also treated with antidepressants) also have short REM latencies. The present work, which made the same observation in panic-anxiety patients when compared to laboratory norms, suggests that it may be meaningful to speak of a range of clinical syndromes characterized by short REM latencies and responding to antidepressants. This could conceivably lead to a new method of clarifying psychiatric disturbances based on biological characteristics.
- 2) This suggests that there is a spectrum of GH regulatory dysfunction, ranging from absolute deficiency to intermittent irregularity of secretion. As will be seen in the accompanying section of this report, the Unit has pursued the issue of sleep-related GH secretion at a basic science level as well.
- 3) Annual alterations in slow-wave sleep are the first major biological marker to clearly be a part of the now well established syndrome of seasonal depressions. It is hoped that this will give more biological meaning to an important clinical conclusion.

Proposed Course:

- 1) We are now performing a pilot study to determine if panic-anxiety patients can be distinguished from normals by excessively disturbed sleep on their "adjustment night" compared to subsequent recordings. Work is in progress to bring in new ages and sex-matched controls for this study.
- 2) This project continues while we build up a larger sample.

3) Circadian studies of sleep are described in Project Z01 MH 02204-01 CP. Among ongoing efforts will be the establishment of a phase response curve of the effect of light pulses on sleep in normals. The clinical effects of a skeleton photoperiod in seasonal depressives will be determined.

Publications:

Rosenthal, N.E., Sack, D.B., Carpenter, C.J., Parry, B., Mendelson, W.B., Wehr, T.A.: Antidepressant effects of light in seasonal depression (submitted).

Mendelson, W.B.: Studies of human growth hormone secretion in sleep and waking. Intl. Rev. Neurobiology, 23:367-389, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02193-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Clinical studies of insomnia		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Wallace B. Mendelson, M.D., Chief, Unit on Sleep Studies		
COOPERATING UNITS (if any) 		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION 		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.3	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The laboratory has had a longstanding interest in <u>insomnia</u>. Last year a major study of the effectiveness and hazards of benzodiazepine use in <u>insomniacs</u> was completed (Z01 MH 02193-01 CP). This year attention was directed to <u>information processing</u> and <u>circadian rhythms</u> in <u>insomniacs</u>. The data suggest that <u>insomniacs</u> may differ from non-complaining individuals in personality traits, cognitive style and temperature regulation. <u>Insomniacs</u> were found to have difficulty retrieving material already well known to them, although there may be no deficits in acquiring new material. They also had temperature curves similar to normals in phase, but about 0.4 F higher. Studies are currently under way to test the hypothesis that, because of heightened mental activity during sleep, <u>insomniacs</u> may perform differently in ability to perceive stimuli and acquire information while asleep. </p>		

Project Description:

This study is designed to examine the difference between insomniacs and persons who are satisfied with their sleep as measured on a variety of physiological tests, measures of sleep, and thinking processes during sleep.

There has been a considerable amount of research on the physiological parameters of insomnia, but relatively little attention has been given to the characteristics of their daytime waking cognitive functioning. Several experiments suggest that insomniacs tend to have a higher level of cerebral arousal during sleep than that of normal persons. This study examined this hypothesis in two ways: (1) by analyzing attentiveness and effortful cognitive processes by day; and (2) examining cognitive processes and perception during sleep.

The second part of the project stems from the observation of Rechtschaffen that when an investigator entered the room of "poor sleepers" who were asleep by EEG criteria (10 minutes past the first sleep spindle), many of the subjects reported that they had indeed already been awake. For this reason, our study examined the experience of being awake and cognitive processes in good and poor sleepers who have been awakened at several points during sleep. Because of interest in the possible contribution of circadian dysrhythmias to insomnia, these patients were also carefully monitored for motor activity and core temperature rhythms.

In summary, hypotheses tested were that self-reported "poor sleepers" differ from "good sleepers" in (1) qualities of daytime attentiveness and effortful cognition; (2) subjective sense of being awake while in EEG-defined State 2 sleep; and (3) EEG sleep stages and measures of biological rhythms.

Findings:

Ten patients with chronic insomnia, in whom sleep apnea, nocturnal myoclonus and major psychiatric disorders have been ruled out, were compared to 10 age and sex-matched controls on a variety of measures. As expected the two groups differed significantly on nine out of nine questions regarding habitual sleep and waking pattern, including total sleep time and daytime tiredness. In contrast, objective measures of sleep (EEG) demonstrated relatively mild alterations in sleep, reaching statistical significance only for increased waking time after sleep onset in insomniacs. Subjects were asked to rate themselves using the Stanford Sleepiness Scale, 100 mm scales of sleepiness, anxiety and energy, and a mood rating quest. In contrast to their description of their habitual patterns, which were quite different, the two groups did not differ on any of these multiple measures of what they were experiencing at any given moment in their waking life. On tests of cognitive function, insomniacs and normals performed similarly in terms of effortful, automatic, and episodic memory functions. In contrast, the insomniacs access to semantic or knowledge memory was significantly poorer than the normals. This latter type of cognitive operation has been shown to determine state dependent learning and retrieval phenomena and may also determine how internal and external events are perceived.

The results of the second part of our project, where patients were awakened by a loud (80 db) tone at 5 points during the night and asked if they were awake or asleep, showed no difference between the groups with the exception of the test during intermittent waking. Insomniacs who reported being asleep, however, tended to describe themselves as being in lighter sleep during EEG defined "deep sleep" in (Stage 4) and significantly more often described lighter sleep in REM, compared to normals.

Each of the 10 insomniacs and normal controls were also given the MMPI. Analysis of the data indicated a significant difference between groups on the F, K, D, and SI scales. Of the total insomniac sample, 60% had one or more of the MMPI scales in the pathological range. As a group, neither insomniacs nor controls showed a significant difference to a pathological degree. Among the more physiologic measures in this study was temperature recording. It was found that during sleep the insomniacs had rhythmic changes in temperature paralleling that of normals, but running about 0.4°C higher.

Significance to Biomedical Research and to the Program of the Institute:

Our findings in insomniacs, notably alterations in the relation of temperature rhythms to sleep, are among the few biological observations in this patient group. Other findings, notably the inability of insomniacs to distinguish between EEG-defined light and deep sleep, may help explain the observation that the subjective complaint of distress of insomniacs is usually greater than EEG-documented disturbance. The principle that a problem of perception of state of consciousness is central to understanding insomnia is being followed up by a new project.

Proposed Course:

A continued analysis of the literature suggests that insomniacs may differ from normal sleepers by the degree of cognitive processes which go on during sleep. It is for this reason that we are presently further exploring arousability and ability to process information during sleep of insomniacs.

In addition to the selection requirements, memory tests, and mood rating scales previously mentioned, the following recording nights have been added to form a new study:

1. Arousal threshold--on one night each, subjects would sleep in the laboratory and be exposed to progressively louder tones (a neutral stimulus) or recordings of an investigator calling their names (a meaningful stimulus), until EEG-defined arousal occurred.
2. While subject is asleep, the investigator will give an auditory suggestion, and classical conditioning procedures will be performed.
3. During different stages of sleep a tone will be presented once a second for six seconds, and the subject will be instructed (before sleep) to push a button taped to his hand when he hears the tone.

Other aspects of this new study will include more detailed observations of temperature rhythms in these patients.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02194-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Anticonvulsant/proconvulsant effects of benzodiazepine receptor ligands		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Wallace B. Mendelson, M.D., Chief, Unit on Sleep Studies		
COOPERATING UNITS (if any) Adult Psychiatry Branch, SMHR, NIMH Laboratory of Preclinical Pharmacology, NIMH Laboratory of Bioorganic Chemistry, NIADDK		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Unit on Sleep Studies		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="text-align: justify;"> Benzodiazepines are thought to have four general types of clinical effects: anxiolytic, muscle relaxant, hypnotic and anticonvulsant. One outgrowth of our previous work with benzodiazepine antagonists and sleep has been an interest in the role of <u>GABA</u> in the proconvulsant and anticonvulsant effects of drugs which bind to the benzodiazepine receptor. A current hypothesis suggested that drugs whose affinity to the receptor is decreased by GABA will be convulsants, while drugs whose affinity is enhanced will be anticonvulsants. This theory would predict that <u>3-carboethoxy-beta-carboline</u> (beta-CCE), would have minimal or no proconvulsant properties, whereas the methoxy derivative (beta-CCM) would be a convulsant. Our laboratory demonstrated that when EEG criteria are used, beta-CCE will indeed induce electroencephalographic responses in rats, and our concurrent biochemical work indicated that the difference in effect can be explained by a faster rate of metabolism of beta-CCE. Similarly, biochemical studies in monkeys showed that the difference in relative resistance of rats to these seizures, and the relative ease of inducing seizures in squirrel monkeys, could be largely explained by rate of metabolism. These studies emphasize the importance of pharmacokinetic considerations as well as the role of GABA in drugs affecting <u>seizure activity</u>. </p>		
(273)		

Other Professional Personnel:

Dr. Richard Wagner	Clinical Associate, Adult Psychiatry Branch, SMHR, NIMH
Dr. M. Corda	Guest Worker, Section on Neuroendocrinology, Laboratory of Preclinical Pharmacology (LPP), NIMH
Dr. Margaret Schweri	Staff Fellow, Section on Pharmacodynamics, Laboratory of Bioorganic Chemistry, NIADDC
Dr. Joseph Martin	Staff Fellow, Unit on Sleep Studies, CPB, NIMH

Project Description:

A. Convulsant action of Beta-CCE in the rat: pharmacokinetic considerations and the role of GABA. Compounds which bind to the benzodiazepine receptor have varying effects on seizure behavior. While "agonists" such as diazepam prevent seizures induced by pentylenetetrazole, "antagonists" such as CGS 8216 block the effect of diazepam. Other compounds, mainly beta-carboline-3-carboxylic acid (BCC) derivatives, have proconvulsant (beta-CCE) or even convulsant (beta-CCM) activities. Braestrup et al. have proposed a separation of the classes of benzodiazepine receptor ligands on the basis of the shift in affinity of the receptor for the ligand when in the presence of GABA. (GABA increases the affinity for agonists [which have anticonvulsant properties] and decreases the affinity for antagonists (convulsants.) We provided an electrophysiological test of this model by determining whether beta-CCE would induce EEG spiking in rats.

B. Convulsant actions of beta carbolines across species (with Dr. Margaret Schweri). Both the methyl ester of beta-carboline-3-carboxylic acid and the 6,7-dimethoxy-4-ethyl derivative of this compound are potent convulsants in rodents, while the ethyl ester of beta-carboline-3-carboxylic acid does not cause motoric convulsions, even when administered at very high doses. As described above, the rate of degradation of these compounds by rat plasma (ex vivo) parallels their potencies as convulsants. We tested beta-CCE for convulsant properties in squirrel monkeys, and measured rates of metabolism in rat and monkey.

Methods:

A. and B. See Methods section of "Studies of the physiology and pharmacology of sleep" (Project Z01 MH 02195-01 CP). Beta-CCE concentrations in the ex vivo plasma experiment were determined by displacement of H³-diazepam in a radioreceptor assay.

Findings to date:

A. We have found that beta-CCE does indeed induce EEG-defined spiking in rats. Plasma studies indicated that the t 1/2 for beta-CCE was approximately 0.78 minutes. In contrast, beta-CCM, which consistently induces seizures in rats, has a t 1/2 of 2.57 minutes.

B. Beta-CCE, as reported previously, induced seizures in the squirrel monkey. Plasma studies showed that in the monkey metabolism of beta-CCE was substantially slower than in the rat.

Significance to Biomedical Research and to the Program of the Institute:

A. and B. The relationship of the benzodiazepine recognition site to the GABA recognition site remains a subject of active investigation among neuropharmacologists. The studies reported here emphasize the importance of considering kinetic factors as well as effects on GABA when evaluating beta-carbolines.

Proposed Course:

We now plan to explore why another beta carboline, FG 7142, has no proconvulsant effects despite a binding response to GABA approximately the same as the convulsant beta-CCE.

Publications:

Mendelson, W.B., Davis, T., Paul, S.M., Skolnick, P.: Do benzodiazepine receptors mediate the anticonflict action of pentobarbital. Life Sci. 32, 2241-2246, 1983.

Schweri, M.M., Martin, J.V., Mendelson, W.B., Barrett, J.E., Paul, S.M., Skolnick, P.: Pharmacokinetic and pharmacodynamic factors contributing to the convulsant action of beta-carboline-3-carboxylic acid esters. (Submitted).

Mendelson, W.B., Gillin, J.C., Wyatt, R.J.: The search for circulating sleep-promoting factors. In Yoshida, H., Hagihara, Y., Ebashi, S. (Eds.): Advances in Pharmacology and Therapeutics II, Vol. 1, CNS Pharmacology Neuropeptides, Pergamon Press, Oxford and New York, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02195-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of the physiology and pharmacology of sleep		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> W. B. Mendelson, M.D., Chief, Unit on Sleep Studies, CP, NIMH		
COOPERATING UNITS (if any) Laboratory of Preclinical Pharmacology, NIMH		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION Unit on Sleep Studies		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5	PROFESSIONAL: 2	OTHER: 3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The animal sleep laboratory has had an active year examining issues including the regulation of sleep, the pharmacology of anxiety and seizures, and approaches to growth hormone regulation. We previously reported that 3-hydroxy-methyl-beta-carboline, which binds to the benzodiazepine receptor and antagonizes the anxiolytic and anticonvulsant effects of diazepam, will induce dose-dependent increases in wakefulness and block the hypnotic actions of flurazepam. We have now given the tertiary butyl ester beta carboline (B-CCT), which is metabolized very slowly, and demonstrated a dose-dependent increase in wakefulness, the time course of which parallels receptor occupancy as measured in our laboratory. This provides further confirmatory evidence that the benzodiazepine receptor may play an important role in physiologic and pharmacologic sleep regulation. Another area of interest stems from the observation that drugs of very different pharmacologic classes may have behaviorally similar anxiolytic properties. In an effort to find some common mode of action, we hypothesized that the benzodiazepine receptor might play a role in the anxiolytic actions of barbiturates. Using Vogel's conflict test, we demonstrated that CGS 8216, a benzodiazepine antagonist, blocked the anxiolytic effects of pentobarbital. In other studies using the Vogel model, it was demonstrated that there are three types of effects on conflict procedures of drugs that bind to the benzodiazepine receptor: anticonflict effects of anxiolytic benzodiazepines, a pro-conflict effect of some beta carbolines, and antagonism of the other two effects by blockers such as CGS 8216 and RO 15-1788. </p> <p> Following up several years of human work on the relationship of sleep to growth hormone (GH) secretion, we studied the effects of sleep deprivation on the ability of GH to stimulate tissue growth (measured by amount of activity of ornithine decarboxylase). Preliminary results indicate that sleep and exercise affect not only the secretion of GH, but also its effectiveness in stimulating growth. </p>		

Other Professional Personnel:

Dr. M. Corda (study IB)
 Dr. Joseph Martin (study III)

Guest Worker
 Staff Fellow

LPP/NIMH
 CP/NIMH

Project Description:

I. Studies on the role of the benzodiazepine receptor in anxiety.

A. Anxiolytic effects of pentobarbital may be mediated by the benzodiazepine receptor. One of the major questions in psychopharmacology is how a variety of compounds of diverse chemical classes can share apparently similar anxiolytic properties. In order to explore the possibility that pentobarbital may produce anxiolytic effects via an influence on the benzodiazepine receptor, we studied whether CGS 8216, a benzodiazepine receptor blocker, might prevent the anxiolytic effects of the barbiturate.

B. Beta-carbolines enhance shock-induced suppression of drinking in rats (with Dr. M. Corda). In another project of this Report (Anticonvulsant/proconvulsant effects of benzodiazepine receptor ligands), we described the issue of the relationship of the benzodiazepine recognition site to the GABA recognition site. In this project we have explored the anxiolytic/anxiogenic properties of compounds whose binding to the benzodiazepine receptor is not influenced (CGS 8216) or reduced (several beta-carbolines) by GABA.

II. Studies of the role of the benzodiazepine receptor in the regulation of sleep.

Studies of drugs which interact with the benzodiazepine receptor have led to the discovery of a series of esters of beta-carboline-3-carboxylic acid which cause behavioral effects that are opposite to the effects of such standard agonists as diazepam. Specifically, these compounds can cause increased wakefulness instead of sedation (Mendelson et al., 1983). The tertiary butyl ester (BCCT) was studied in greater detail since the hydrolysis of this compound is very slow in plasma.

III. The relationship of sleep to effectiveness of growth hormone (GH) in stimulating tissue growth.

This Unit has had a long-standing interest at a clinical level in the mechanism of release of growth hormone (see Project Z01 MH 02200-01 CP, "Sleep in psychiatric and endocrine disorders"). One hypothesis we developed is that the state of consciousness will influence the ability of GH to stimulate tissue growth. We have pursued this question at a basic science level by studying whether sleep deprivation will affect growth hormone stimulation of ornithine decarboxylase activity (a measure of cell reproduction rate).

Methods:

I. As a measure of anxiolytic/anxiogenic properties of compounds, we employed the conflict model of Vogel. In essence, water-deprived rats are placed in a situation where they are shocked after every three seconds of

accumulated drinking time. Compounds which are anxiolytic increase the number of shocks an animal is willing to accept. By reducing the shock level we have modified the test so that anxiogenic effects are also manifested (as a decrease in number of shocks received).

II. Sleep EEG is recorded from rats following implantation of stainless steel screw dural electrodes. Electromyogram is taken from stainless steel wire electrodes implanted in the neck strap muscles. The state of consciousness (waking, non-REM, REM sleep) is determined for every 30 second shock of the recording.

III. Following sleep deprivation (by running on a slow treadmill) or sham procedure, rats were injected with bovine GH. At hourly intervals thereafter animals were killed, and hepatic ornithine decarboxylase activity was measured by a CO₂ capture technique.

Findings to date:

IA. The benzodiazepine receptor blocker CGS 8216 (2.5-10 mg/kg i.p.) did not affect nonpunished responding, but doses of 5 and 10 mg/kg significantly reduced the rate of punished responding (i.e., the number of 3 second drinking episodes in a "shock" contingency). However, a dose of CGS 8216 which did not significantly alter punished responding (2.5 mg/kg) antagonized the anticonflict actions of pentobarbital. These observations suggest that while high doses of CGS 8216 may elicit an "anxiogenic" response in rodents, lower doses of CGS 8216 antagonize the anticonflict actions of a compound which has been shown to enhance benzodiazepine affinity in vitro. These data imply that the anticonflict actions of pentobarbital may be mediated through benzodiazepine receptors.

IB. By using Vogel's method to test the anxiolytic action of benzodiazepines and reducing the intensity of the current delivered to the drinking tube, it was found that one could distinguish the pharmacological activity of three types of ligands for the benzodiazepine recognition site: an anticonflict action typical of anxiolytic benzodiazepines, a proconflict action typical of many beta-carbolines, including FG 7142 (beta-carboline-3-carboxylic acid ethyl ester methyl amide), and an antagonistic action of the proconflict and anticonflict actions typical of RO 15-1788 (ethyl-8-fluoro-5, 6-dihydro-5-methyl-6-oxo-4H-imidazol [1,5-a]-[1,4]-benzodiazepine-3-carboxylate) and CGS 8216 (2-phenyl-pyrazolo [4,3-c] quinolin-3-(5H)-one). Pentylenetetrazole, which causes convulsions by interacting with a subunit of the GABA receptor that is different from the benzodiazepine recognition site, also induces a proconflict action that is antagonized by anxiolytic benzodiazepines but not by RO 15-1788.

II. While BCCT was found to cause a marked decrease in sleep at 1 hr after injection, 5 hrs later sleeping occurred normally. Similarly, at 1 hr after BCCT injection the in vivo binding of ³H diazepam was greatly reduced, presumably since BCCT occupied the receptor site. In vitro studies indicated an increase in K_d but not B_{max}, which would indicate competitive inhibition of the diazepam binding. At six hours after BCCT injection, both in vivo and in vitro binding of [³H] diazepam had returned to control (pre-injection) levels, and we believe the BCCT had been excreted by this time.

III. Tentative data from the study suggests that the sleep-deprived group had heightened ornithine decarboxylase response to GH.

Significance to Biomedical Research and to the Program of the Institute:

IA. The study, which found that the anxiolytic properties of pentobarbital are prevented by pretreatment with a benzodiazepine receptor blocker, suggests that the anxiolytic effects of some non-benzodiazepines may occur via influencing some aspect of the receptor. This is a step toward a more unitary view of the actions of anxiolytics of diverse pharmacologic classes.

IB. These studies relate type interaction of the benzodiazepine recognition site to the GABA recognition site to the ability of ligands to have anxiolytic or anxiogenic properties.

II. The work, which shows a parallelism between benzodiazepine receptor occupancy by a beta-carboline and its ability to induce wakefulness, provides further evidence that this receptor site mediates pharmacologic sleep induction by benzodiazepines.

III. It has been known for some time that the state of consciousness has an important role in the release of GH. These new data suggest that sleep or wakefulness (or some aspect of the sleep deprivation procedure) also affects the ability of GH (once secreted) to stimulate tissue growth.

Proposed Course:

IA. We are now studying whether a benzodiazepine receptor blocker will prevent sleep induction by pentobarbital.

IB. We are currently exploring the effects on the conflict model of drugs which influence the "peripheral" benzodiazepine receptor, which is now known to be found in the CNS also.

II. Having studied a variety of beta-carbolines on sleep, we are now looking at the effects of other classes of benzodiazepine antagonists.

III. We are continuing to build a larger "n" in this study of growth hormone.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02204-01 CP
PERIOD COVERED October 1, 1982, through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of sleep as a circadian rhythm		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Wallace B. Mendelson, M.D., Chief, Unit on Sleep Studies		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Psychobiology Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">1.5</div>	PROFESSIONAL: <div style="text-align: center;">0.3</div>	OTHER: <div style="text-align: center;">1.2</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: justify; padding: 10px;"> <p>The Unit has conducted a series of projects to elucidate the rhythmic aspects of sleep, and has also participated in broader studies of <u>biological rhythms</u> in <u>depressive illness</u>. In order to determine if the circadian rhythms of <u>temperature</u> and <u>sleep</u> represent "the hands of the clock" or whether they interact on some more fundamental level, studies of sleep are being performed on subjects wearing a "<u>space suit</u>" on loan from NASA. Using control systems developed here, the suit can virtually abolish the circadian rhythm of temperature. In another project normals and depressed patients are placed on a 1 hour dark/2 hour light schedule to enhance the rhythmic aspects of REM sleep and determine if depressives are phase advanced in this measure.</p> </div>		

Other Professional Personnel:

Thomas A. Wehr, M.D.

Acting Chief

CP/NIMH

Project Description:

Biological rhythms in rapidly changing light-dark cycles. One way to enhance the appearance of circadian qualities of sleep is to allow subjects to sleep briefly in multiple episodes around the circadian day. In this study, normal volunteers (n=6) were put on a schedule of 1 hour dark: 2 hour light for 48 hours. A clear circadian rhythm of REM sleep emerges under these conditions, with a peak propensity for appearance at about 6 a.m. Studies are now being performed in depressed individuals to determine if there is any phase advance in their REM rhythm.

Effects of control of core temperature on rhythmic aspects of sleep. The Unit has developed a system for regulating core temperature by use of a space suit obtained from NASA. The goal of this project is to examine sleep during a 24-hour period in which core temperature is held constant. Data from this technique may help determine whether the rhythms of sleep and temperature are both "hands of the clock" or whether they interact in some more fundamental fashion. The development phase of this project is just being completed, and formal data acquisition is beginning.

Methods:

See "Methods" section of "Sleep in Psychiatric and Endocrine Disorders" (Project Z01 MH 02192-01 CP).

Findings:

Data have so far been collected from six controls and four depressed patients. In the controls it became clear that this technique does indeed enhance the manifestation of circadian aspects of REM sleep. Amounts of REM sleep clearly peak at about 6 a.m. Among the four depressed patients, two showed a shift of REM sleep more toward 3 a.m.

The past months have been spent developing and testing the "space suit" controls and heat exchange. In June of this year this development stage was completed, and temperature has been held constant over 24 hours in three subjects. Formal data acquisition for the main study is now beginning.

Significance to Biomedical Research and to the Program of the Institute

The phase advance hypothesis of depression is a major, testable principle in biological psychiatry. The "three hour day" procedure, by bringing out the innate REM rhythm, will provide an important measure of REM rhythmicity in depression.

That there is some relationship between sleep and temperature rhythms appears evident from temporal isolation studies. The "space suit" procedure,

which controls one of these two rhythms, may provide insight into the nature of this relationship, i.e., does one drive the other, or are they both secondary to some more fundamental mechanism.

Proposed Course:

We will continue to build a higher "n". An additional effort will be made to find what characteristics separate depressives who show a phase advance from those who do not.

The data acquisition phase of this project is now beginning, and will continue to be actively performed this year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00422-12 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuropharmacology of Circadian Rhythms		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Joseph S. Takahashi, Research Associate, LCS, NIMH		
COOPERATING UNITS (if any) Department of Biology, University of Houston, Houston, Texas		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: center; margin-top: 100px;"> TERMINATED </div>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00425-07 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Peripheral and Central Catecholamines in Hypertension and Stress		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Juan M. Saavedra, Medical Officer (Psychiatry), LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH; Clinical Psychobiology Branch, NIMH; Laboratory of Psychobiology, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center; font-size: 1.2em;">5.0</div>	PROFESSIONAL: <div style="text-align: center; font-size: 1.2em;">5.0</div>	OTHER: <div style="text-align: center; font-size: 1.2em;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 10px 0;"> This project was initiated to study the role of <u>catecholamines</u> in the regulation of <u>hypertension</u> and <u>stress</u>, and was later extended to study the participation of other <u>biogenic amines</u>, <u>estrogens</u> and <u>prostaglandins</u> in the central regulation of <u>neurovegetative functions</u>. </p> <p style="margin: 10px 0;"> <u>Catecholamines</u> in <u>postero-intermediate pituitary</u> present a circadian rhythm and are regulated by <u>estrogen receptors</u>. </p> <p style="margin: 10px 0;"> In addition to catecholamines, <u>serotonin</u> and <u>histamine</u> participate in the <u>central regulation</u> of <u>cardiovascular function</u>. </p> <p style="margin: 10px 0;"> There are two distinct enzymes in the brain which are capable of synthesizing <u>epinephrine</u>. These enzymes are not connected anatomically. </p> <p style="margin: 10px 0;"> In addition to <u>hypothalamic</u> and <u>brain stem catecholamines</u>, <u>septal catecholamines</u> participate in the central <u>stress</u> response. </p>		

Names, Laboratory and Institute Affiliations, and Titles of All Other Professional Personnel Engaged on the Project.

Others:	Henry H. Holcomb, Clinical Associate	LP	NIMH
	Paul M. Gross, Visiting Fellow	LCM	NIMH
	Masako Kadekaro, Visiting Scientist	LCM	NIMH
	Louis Sokoloff, Chief	LCM	NIMH
	Markku Koulo, Visiting Fellow	CPB	NIMH
	Markku Linnoila, Chief, Clinical Studies	CPB	NIMH

Projection Description:

Objectives: To study the role of central and peripheral catecholamines, other biogenic amines and prostaglandins in hypertension and stress.

Methods Employed: Neuroanatomical, surgical, biochemical.

Major Findings:

Biogenic amines in pituitary gland - There is a circadian rhythm in catecholamines in postero-intermediate lobe. 17- β -Estradiol administration increases postero-intermediate catecholamine levels and turn-over in ovariectomized rats, an effect which is most prominent in the intermediate lobe.

Brain biogenic amines - Specific septal and preoptic areas show large changes in catecholamines after stress. Two different adrenaline-forming enzymes occur in brain. Brain stem serotonin is involved in the regulation of blood pressure.

Peripheral catecholamines in hypertension - Specific alterations in kidney, adrenal and plasma catecholamines occur in genetically hypertensive rats and in hypertensive rats genetically susceptible to high salt diet.

Significance to Biomedical Research: These studies could help to explain some aspects of the central regulation of cardiovascular functions and the pathology of hypertension, as well as the role and regulation of peripheral sympathetic nerves and adrenal medulla in hypertension and stress.

A better understanding of the role of the brain and the sympathetic system in these phenomena could result in the development of new drugs with a predominant central effect which could be of use in the treatment of diverse psychosomatic illnesses related to pathological stress or to disorders of cardiovascular regulation.

Proposed Course of Project: Further studies will be conducted to analyze the role of estrogens on pituitary catecholamines and the physiological implications of the circadian rhythms of catecholamines in the pituitary gland.

The role of brain catecholamines, serotonin and other biogenic amines in stress and blood pressure regulation will be studied with emphasis on the simultaneous determination of levels of amines and amine metabolites, and turn-

over studies.

Publications:

Barden, N., Chevillard, C., and Saavedra, J.M.: Effects of acute and chronic 17- β -estradiol administration on rhombencephalic, pineal and pituitary catecholamine levels in ovariectomized rats. Neuroendocrinology 35: 123-127, 1982.

Fernandez-Pardal, J., and Saavedra, J.M.: Catecholamines in discrete kidney regions. Changes in salt-sensitive Dahl hypertensive rats. Hypertension 4: 821-826, 1982.

Grobecker, H., Saavedra, J.M., and Weise, V.K.: Biosynthetic enzyme activities and catecholamines in adrenal glands of genetic and experimental hypertensive rats. Circ. Res. 50: 742-746, 1982.

McCarty, R., and Saavedra, J.M.: Plasma catecholamines in salt-sensitive hypertensive (Dahl) rats. Physiol. Behav. 28: 1083-1088, 1982.

Pettinger, W.A., Gandler, T., Sanchez, A., and Saavedra, J.M.: Dietary sodium and renal α_2 -adrenergic receptors in Dahl hypertensive rats. Clin. Exp. Hypertens. A4 (4 and 5): 819-828, 1982.

Saavedra, J.M.: Changes in dopamine, noradrenaline and adrenaline in specific septal and preoptic nuclei after acute immobilization stress. Neuroendocrinology 35: 396-401, 1982.

Saavedra, J.M., Barden, N., Chevillard, C., and Fernandez-pardal, J.: Twenty-four-hour rhythm and effects of stress and adrenalectomy on rat pineal, noradrenaline and adrenaline concentrations. Cell. Molec. Neurobiol. 2: 1-10, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00428-04 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983.		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Protein Carboxyl Methylation: A Post Translational Modifier of Protein Function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Juan M. Saavedra, Medical Officer (Psychiatry), LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH ADAHMA NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">1.0</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Enzymatic carboxyl methylation</u> of proteins is a post translational modification. <u>Carboxyl methylation</u> reduces negative charges on <u>proteins</u> and alters their structure and properties, providing an important regulatory mechanism. </p> <p> Protein carboxyl methylation is associated with <u>exocytotic release</u>, the latter dependent on <u>depolarization</u> of the cell membrane and Ca^{++} movements. Posterior pituitary <u>hormones</u> <u>vasopressin</u> and <u>oxytocin</u> and their corresponding neurophysins are released by <u>exocytosis</u> which occurs upon <u>depolarization</u>. </p> <p> The <u>homozygous Brattleboro rat</u> lacks <u>vasopressin</u> and its associated neurophysin, and presents the syndrome of <u>diabetes insipidus</u>. Both homogenates of <u>posterior pituitary</u> and whole posterior lobes incubated <u>in vitro</u> show a large decrease in endogenous methyl acceptor proteins in homozygous Brattleboro rats. Physiological and biochemical studies demonstrated that most of the decrease in <u>endogenous methyl acceptor proteins</u> in these animals is the result of the absence of <u>vasopressin-associated neurophysin</u>. <u>Protein carboxyl methylase</u> activity is high in <u>homozygous Brattleboro rats</u>, a change probably correlated with hyperactivity of the <u>neurosecretory posterior pituitary</u> system. These data indicate a close association between protein carboxyl methylation and <u>posterior pituitary neurosecretion</u>. In addition, other, still unidentified proteins occur as <u>endogenous methyl acceptor</u> proteins in the rat <u>posterior pituitary</u>. </p>		

Names, Laboratory and Institute Affiliations, and Titles of Co-principal Investigator and All Other Professional Personnel Engaged on the Project.

Co-principal Investigator:

Julius Axelrod, Chief, Section on Pharmacology

LCS NIMH

Others: Henry H. Holcomb, Clinical Associate

LPP NIMH

Project Description:

Objectives: To study the relationship between enzymatic carboxyl methylation and exocytotic release, and to identify newly detected endogenous methyl acceptor proteins in pituitary gland.

Methods Employed: Enzymatic, pharmacologic, gel electrophoresis, HPLC, RIA.

Major Findings: Whole rat posterior pituitary lobes incubated in the presence of [^3H]-methionine enzymatically methylate at least 6 distinct proteins, one of them neurophysin. The identity of the other 5 proteins is presently unknown; however, at least 3 of these are located in neurosecretory axons originated in the hypothalamus. Homozygous Brattleboro rats lacking vasopressin-neurophysin and presenting the syndrome of diabetes insipidus showed decreased carboxyl methylation in whole posterior pituitaries in vitro.

Significance to Biomedical Research: We have presented evidence on alterations in protein carboxyl methylation in a disease, diabetes insipidus. Carboxyl methylation, by reducing negative charges on membrane proteins, may prove to be a key regulatory step during exocytosis.

Proposed Course of Project: We will attempt to correlate changes in protein carboxyl methylation in pituitary gland with alterations in the exocytotic release of neurophysins and related proteins. We will also attempt to identify the newly discovered endogenous methyl acceptor proteins in pituitary glands.

Publications:

Kloog, Y., Axelrod, J., and Spector, I.: Protein carboxyl methylation increases in parallel with differentiation of neuroblastoma cells. J. Neurochem. 40: 522-529, 1983.

Kloog, Y., and Saavedra, J.M.: Protein carboxyl methylation in intact rat posterior pituitary lobes in vitro. J. Biol. Chem., 1983, in press.

Saavedra, J.M., Kloog, Y., Chevillard, C., and Fernandez-Pardal, J.: High protein carboxymethylase activity and low endogenous methyl acceptor proteins in posterior pituitary lobe of rats lacking neurophysin-vasopressin. J. Neurochem., 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00429-04 LCS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biosynthesis of Nonpolar Methylated Lipids

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Martin Zatz, Medical Officer (Research), LCS, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Pharmacology

INSTITUTE AND LOCATION

NIMH ADAMHA NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.3

PROFESSIONAL:

1.0

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

TERMINATED

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00433-03 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Neuropeptides in Neuroendocrine Regulation		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Juan M. Saavedra, Medical Officer (Psychiatry), LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Cerebral Metabolism, NIMH; Laboratory of Psychology and Psychopathology, NIMH; Institute Pasteur, France		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH ADAMHA NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">5.0</div>	PROFESSIONAL: <div style="text-align: center;">5.0</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project was initiated to study the role of <u>brain neuropeptides</u> in <u>genetic hypertension</u> and <u>stress</u> and was later extended to study the <u>inter-relationship</u> between different neuropeptides and their role in <u>neuroendocrine regulation</u>. </p> <p> We took advantage of <u>animal models</u> of disease: the <u>spontaneously hypertensive rat</u>, which in its <u>homozygous</u> state lacks the <u>antidiuretic hormone vasopressin</u>, and presents the syndrome of <u>diabetes insipidus</u>. </p> <p> <u>Spontaneously hypertensive rats</u> show alterations in <u>angiotensin-converting enzyme</u> in peripheral tissues and specific <u>forebrain nuclei</u>. </p> <p> Changes in <u>somatostatin metabolism</u> and in the activity of <u>angiotensin-converting enzyme</u> occur in <u>pituitary gland</u> and <u>specific brain nuclei</u> of <u>Brattleboro</u> rats. </p> <p> Endogenous <u>somatostatin</u> occurs in the <u>pituitary gland</u>, where it is probably present in <u>nerve terminals</u> to the <u>intermediate lobe</u> which originates in the <u>hypothalamus</u>. In the <u>intermediate lobe</u>, <u>somatostatin</u> inhibits the <u>iso-proterenol-stimulated adenylate cyclase</u>, and it is probably involved in the release of <u>neuropeptides</u> from this structure. </p>		

Names, Laboratory and Institute Affiliations, and Titles of All Other Professional Personnel Engaged on the Project.

Others:	Claude Chevillard, Guest Worker	LCS	NIMH
	Fernand Dray, Head, Unit on RIA, Institute Pasteur, France		
	Kyriaki Gerozitssis, Established Investigator, Unit on RIA, Institute Pasteur, France		
	Catherine Rougeot, Research Assistant, Unit on RIA, Institute Pasteur, France		
	Henry H. Holcomb, Clinical Associate	LPP	NIMH
	Paul M. Gross, Visiting Fellow	LCM	NIMH
	Masako Kadekaro, Visiting Scientist	LCM	NIMH
	Louis Sokoloff, Chief	LCM	NIMH

Project Description:

Objectives: To study the functions of central neuropeptides, their role in neuroendocrine regulation, and the interactions between different neuropeptide systems.

Methods Employed: Neuroanatomical, biochemical, RIA, HPLC.

Major Findings:

Brain peptidases - Alterations in angiotensin-converting enzyme (kininase II) activity occur in forebrain areas of spontaneously hypertensive rats and in extrahypothalamic areas of Brattleboro rats lacking vasopressin.

Brain and pituitary neuropeptides - There is endogenous somatostatin in the posterior and especially in the intermediate pituitary, where this peptide could play a role by regulating the β -adrenergic receptor involved in hormone secretion. There are specific, vasopressin-reversible changes in somatostatin in rats lacking vasopressin (Brattleboro rats). These changes are localized to the hypothalamic periventricular somatostatin system.

Significance to Biomedical Research: The study of the interactions between different neuropeptides will partially clarify the role of these compounds in neuroendocrine regulation. Drugs could be developed which by modulating neuropeptide systems would be of future therapeutic use in the treatment of neuroendocrine disorders.

Proposed Course of Project: We plan to study further the interactions and roles of brain and pituitary neuropeptides, with special emphasis on vasopressin, angiotensin and somatostatin.

Publications:

Chevillard, C., and Saavedra, J.M.: Angiotensin-converting enzyme (kininase II) in pituitary gland of spontaneously hypertensive rats. Regul. Pept., 1983, in press.

Chevillard, C., and Saavedra, J.M.: Selective increase of angiotensin-converting enzyme activity in discrete extrahypothalamic areas of Brattleboro rats. Brain Res., 1983, in press.

Correa, F.M.A., and Saavedra, J.M.: Somatostatin inhibits the isoproterenol-stimulated adenylate cyclase in the intermediate lobe of the male rat pituitary gland. Neuroendocrinology, 1983, in press.

Saavedra, J.M., and Chevillard, C.: Vasopressin-reversible increase in angiotensin-converting enzyme in specific hypothalamic nuclei of Brattleboro rats. Brain Res. 246: 157-160, 1982.

Saavedra, J.M., Fernandez-Pardal, J., and Chevillard, C.: Angiotensin-converting enzyme in discrete areas of the rat forebrain and pituitary gland. Brain Res. 245: 317-325, 1982.

Saavedra, J.M., Rougeot, C., Chevillard, C., and Dray, F.: High, vasopressin-reversible, immunoreactive somatostatin in hypothalamic nuclei of rats with diabetes insipidus (Brattleboro rats). Brain Res., 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00434-02 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cellular Mechanisms of ACTH Secretion from Mouse Pituitary Tumor Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Julius Axelrod, Chief, Section on Pharmacology, LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Cell Biology, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Pharmacology		
INSTITUTE AND LOCATION NIMH ADAMHA NIH Bethesda, Maryland 20205		
TOTAL MANYEARS: 3	PROFESSIONAL: 3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Using AtT-20/D16-16 mouse pituitary cells rich in corticotropins we found that there are many hormones that can stimulate the release of ACTH. These are corticotropin releasing factor (CRF), catecholamines and vasoactive intestinal peptide (VIP). Catecholamines release ACTH via a β_2-adrenergic receptor mechanism. The receptor mediated release of ACTH by these hormones act by generating cyclic AMP. ACTH liberates glucocorticoids which, in turn, inhibit the CRF, catecholamines and VIP mediated release of ACTH in AtT-20/D16-16 cells. Somatostatin also blocks the release of the receptor mediated release of ACTH by blocking the action of adenylate cyclase.</p> <p>Pretreatment of AtT-20/D16-16 cells with the β-adrenergic agonist markedly reduces the release of ACTH by catecholamines. A similar desensitization of ACTH release by CRF in primary pituitary cells was found. A desensitization of the inhibitory effects of somatostatin to receptor mediated ACTH was observed. We have found that isoproterenol can cause the secretion of ACTH in the rat by directly stimulating the β_2-adrenergic receptors on the anterior pituitary. The catecholamine stimulated release of ACTH <u>in vivo</u> can be blocked by glucocorticoids. These observations show that the three stress hormones, catecholamines, ACTH and glucocorticoids, are interrelated and should have important implications in understanding different types of stress.</p>		
(301)		

Names, Laboratory and Institute Affiliations, and Titles of Co-principal Investigator and All Other Professional Personnel Engaged on the Project.

Co-principal Investigator:

Terry D. Reisine, Guest Worker

LCS NIMH

Others: Vivian Y.H. Hook, Staff Fellow

LCB NIMH

Eva Mezey, Visiting Fellow

LCB NIMH

Miklos Palkovits, Visiting Scientist

LCB NIMH

Projection Description:

Objectives: To investigate at the cellular and molecular level various aspects of the ACTH secretory pathway in the mouse clonal pituitary tumor cell line, AtT-20/D16-16. The role of catecholamines in the release ACTH in various stresses will be examined.

Methods Employed:

Cell culture. Biochemical: Radioimmunoassay of ACTH, and cyclic nucleotides; gel electrophoresis, enzyme assays, cellular subfractionation and characterization. Pharmacological: Radioreceptor assays.

Major Findings:

Using AtT-20/D16-16 mouse pituitary cells rich in corticotropins we found that there are many hormones that can stimulate the release of ACTH. These are corticotropin releasing factor (CRF), catecholamines and vasoactive intestinal peptide (VIP). Catecholamines release ACTH via a β_2 -adrenergic receptor mechanism. The receptor mediated release of ACTH by these hormones act by generating cyclic AMP. ACTH liberates glucocorticoids which, in turn, inhibit the CRF, catecholamines and VIP mediated release of ACTH in AtT-20/D16-16 cells. Somatostatin also blocks the release of the receptor mediated release of ACTH by blocking the action of adenylate cyclase.

Pretreatment of AtT-20/D16-16 cells with the β -adrenergic agonist markedly reduces the release of ACTH by catecholamines. A similar desensitization of ACTH release by CRF in primary pituitary cells was found. A desensitization of the inhibitory effects of somatostatin to receptor mediated ACTH was observed. We have found that isoproterenol can cause the secretion of ACTH in the rat by directly stimulating the β_2 -adrenergic receptors on the anterior pituitary. The catecholamine stimulated release of ACTH *in vivo* can be blocked by glucocorticoids. These observations show that the three stress hormones, catecholamines, ACTH and glucocorticoids, are interrelated and should have important implications in understanding different types of stress.

Significance to Biomedical Research: The study of the cellular and molecular interactions of different peptides and catecholamines on the AtT-20 cell will clarify the importance of these substances in the pathogenesis of stress-induced ACTH release.

Proposed Course of Project: We plan to examine molecular mechanisms of the release of ACTH and the inhibitory effects of somatostatin. Also to be studied is the role of β_2 -adrenergic receptors in the release of ACTH from the pituitary and the effects of various types of stress.

Publications:

Heisler, S., Reisine, T.D., and Axelrod, J.: Desensitization of β_2 -adrenergic receptors and adrenocorticotropin release. Biochem. Biophys. Res. Commun. 111: 112-119, 1983.

Heisler, S., Reisine, T.D., Hook, V.Y.H., and Axelrod, J.: Somatostatin inhibits multireceptor stimulation of cyclic AMP formation and corticotropin secretion in mouse pituitary tumor cells. Proc. Natl. Acad. Sci. USA 79: 6502-6506, 1982.

Hook, V.Y.H., Heisler, S., and Axelrod, J.: Corticotropin-releasing factor stimulates phospholipid methylation and corticotropin secretion in mouse pituitary tumor cells. Proc. Natl. Acad. Sci. USA 79: 6220-6224, 1982.

Hook, V.Y.H., Heisler, S., Sabol, S.L., and Axelrod, J.: Corticotropin releasing factor stimulates adenocorticotropin and β -endorphin release from AtT-20 mouse pituitary tumor cells. Biochem. Biophys. Res. Commun. 106: 1364-1371, 1982.

Reisine, T.D., Heisler, S., Hook, V.Y.H., and Axelrod, J.: Multi-receptor-induced release of adrenocorticotropin from anterior pituitary tumor cells. Biochem. Biophys. Res. Commun. 108: 1251-1257, 1982.

Reisine, T.D., Heisler, S., Hook, V.Y.H., and Axelrod, J.: Activation of β_2 -adrenergic receptors on mouse anterior pituitary tumor cells increases cyclic adenosine 3':5'-monophosphate synthesis and adrenocorticotropin release. J. Neurosci. 3: 725-732, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00382-09 LCS

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Localization and Characterization of Brain Neuropeptides

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

David M. Jacobowitz, Chief, Section on Histopharmacology, LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Histopharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.8

PROFESSIONAL:

1.4

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Immunohistochemical studies revealed a wide distribution of pancreatic polypeptide (PP) immunoreactive cell bodies and nerve fibers in the rat central and peripheral nervous systems. A number of PP-immunoreactive cells were demonstrated to coexist with catecholamine neurons in the pons-medulla and sympathetic ganglia. Within the superior cervical ganglia, PP was found to coexist with norepinephrine in a subpopulation of the catecholamine neurons. This subpopulation of neurons was restricted to those innervating the blood vessels of the head and neck. Following electrical stimulation of the cervical sympathetic trunk, PP was coreleased with norepinephrine from the nerves. An immunohistochemical study of PP neurons in the monkey brain revealed a widespread innervation similar to that in the rat.

Corticotropin releasing factor (CRF) was revealed in the rat CNS using an antibody to synthetic ovine CRF as an immunohistochemical marker. CRF immunoreactivity was observed in the hypothalamus, thalamus, amygdala, cerebral cortex, midbrain, pons, medulla and spinal cord. CRF was not observed in the peripheral nervous system.

Immunohistochemical studies of γ_3 -MSH revealed a distribution of immunoreactive neurons limited to the arcuate nucleus and basomedial hypothalamus. These cells were demonstrated to contain other neuropeptides (α -MSH, β -endorphin, ACTH) also derived from pro-opiomelanocortin.

Project Description:

Other Professional Personnel:

John Olschowka	Staff Fellow	LCS NIMH
Debra Diz	Guest Worker (NIGMS Fellow)	LCS NIMH NIGMS

Objectives: 1) Identification and localization of pancreatic polypeptide-like immunoreactive cell bodies and nerve fibers in the central and peripheral nervous systems of rat and rhesus monkey; investigate the possible coexistence and co-release of PP from catecholamine neurons of the rat peripheral nervous system. 2) Identification of corticotropin releasing factor-like immunoreactive cell bodies and nerve fibers in the rat CNS; determine if CRF coexists with other known neurotransmitters. 3) Identification of γ -melanocyte stimulating hormone (γ -MSH) immunoreactive neurons in the rat CNS; determine if γ -MSH coexists with other neuropeptides also derived from the large precursor pro-opiomelanotropin.

Methods Employed: 1) Production of antibodies to prepared neuropeptide-thyroglobulin conjugates; 2) Immunohistochemistry of PP, CRF and γ -MSH; 3) Electrical stimulation of peripheral sympathetic nerves of the rat; 4) Stereotactically-placed microinjections of retrogradely transported fluorescent dyes.

Major Findings:

(A) Pancreatic Polypeptide. 1) Distribution of PP immunoreactive neurons and nerve fibers in the central nervous system. In the previous year, we described a widespread distribution of PP cell bodies and nerve fibers throughout the rat CNS. A similar analysis of rhesus brain also revealed PP neurons throughout the CNS. Cell bodies were observed in the cerebral cortex, hippocampus, caudate, preoptic area and hypothalamus. As in the rat, the largest number of PP neurons were observed in the arcuate nucleus. Cell bodies were also found in the anterior and intermediate lobes of the pituitary. Unlike the rat, which was colchicine treated, no PP cells were observed in the brainstem or spinal cord. PP nerve fibers were widely distributed, but were especially dense in the following nuclei: accumbens, interstitialis stria terminalis, suprachiasmaticus, periventricular thalamic and hypothalamic, paraventricular, dorsomedialis, parabrachialis dorsalis, tractus solitarius, substantia gelatinosa trigemini and dorsal horn of the spinal cord. 2) Coexistence of PP and catecholamine in neurons of the superior cervical ganglia (SCG). Adjacent cryostat sections of the SCG stained by the indirect immunofluorescence technique for either PP or dopamine- β -hydroxylase (DBH) revealed that virtually all (90-95%) SCG cells contained DBH and a subpopulation (30-50%) also contained PP. Ligation of the pre- and postganglionic nerves of the SCG demonstrated PP fibers emanating from the SCG via both axonal trunks. This subpopulation of SCG neurons with both PP and catecholamine was limited to innervating the vasculature of the head and neck. 3) Corelease of PP and catecholamine from SCG nerve fibers. Norepinephrine has previously been shown to be released from SCG nerve fibers by electrically stimulating its preganglionic nerve. A similar release of PP was observed by electrical stimulation of the preganglionic cervical sympathetic nerve trunk.

While this suggests a corelease of the two transmitters, their mechanisms of storage and release from the nerve terminal appear different, for PP was not effected by reserpine.

(B) Corticotropin Releasing Factor. 1) Distribution of CRF immunoreactive neurons and nerve fibers in the rat central nervous system. The antibody to CRF was raised in rabbits against synthetic ovine CRF coupled to bovine thyroglobulin. This antibody, using the indirect immunofluorescence technique, revealed CRF neurons and fibers widely distributed in rat brain. Large numbers of CRF perikarya were observed in the nucleus paraventricularis, with scattered cells in the following nuclei: interstitialis stria terminalis, preopticus medialis, supraopticus, amygdaloideus centralis, dorsomedialis, parabrachialis dorsalis and ventralis, tegmenti dorsalis lateralis, tractus solitarius and reticularis lateralis. CRF nerve fibers were observed in the cerebral cortex, thalamus, hypothalamus, amygdala, midbrain, pons, medulla and spinal cord. These findings suggest that CRF may be involved in a neurotransmitter/neuromodulator role, as well as a hypophysiotropic role. 2) The coexistence of CRF, substance P (SP) and acetylcholinesterase (AChE) in the nucleus tegmenti dorsalis (ntdl). After mapping CRF in the rat brain, it was noted that the patterns of terminal arborization and location of cell bodies for CRF were similar to those for SP in a number of regions. The cell bodies of the ntdl are particularly interesting for they have been shown to contain AChE and project to the medial frontal cortex, septum and thalamus. To determine the coexistence of CRF, SP and AChE, cryostat sections of the ntdl were first stained for CRF and photographed. The CRF antiserum was then removed by elution and the section restained for SP. Alternatively, sections were first stained for AChE and then SP. CRF and SP were found to completely coexist in ntdl neurons. AChE histochemistry revealed a larger number of neurons within the ntdl, a portion of which contained SP/CRF. These CRF-SP-AChE cells were subsequently found to project to the medial frontal cortex, septum and thalamus. Following injection of fluorescent dyes in these regions, labeled cells in the ntdl were observed to also contain CRF, SP or AChE.

(C) γ -Melanocyte Stimulating Hormone. 1) Distribution of γ -MSH immunoreactive neurons and nerve fibers in the rat brain. γ -MSH immunoreactive perikarya were observed only in the arcuate nucleus and basomedial hypothalamus. These cells were identified projecting to the following nuclei: septal, amygdaloid, interstitialis stria terminalis, medial preoptic, anterior hypothalamic, periventricular, paraventricular, arcuate, dorsomedial, posterior hypothalamic, central gray and parabrachial. 2) Coexistence of γ -MSH with pro-opiomelanocortin-derived neuropeptides. The distribution of γ -MSH was remarkably similar to that of α -MSH and β -endorphin. To determine if these neuropeptides all coexist in the same cell, cryostat sections of rat arcuate nucleus were first stained for γ -MSH and photographed. The antiserum was then removed by elution with acidified KMnO_4 and restained for α -MSH or β -endorphin. On occasion, the section was stained a third time to demonstrate all three neuropeptides. The results suggested that all three of these neuropeptides were located in the same cells. The physiologic significance of this coexistence is not known.

Significance to Biomedical Research and the Program of the Institute: The basic neuroanatomical and physiological studies reported here lay the groundwork

for a rational approach to studying the role of pancreatic polypeptide, corticotropin releasing factor and γ -melanocyte stimulating hormone in central and peripheral nervous system physiology.

Proposed Course of the Project:

1) Attempts are in progress to develop a radioimmunoassay for PP in order to determine the biochemical levels of PP within various regions of the brain. Biochemical levels will be determined for regeneration studies, drug treatment studies and stress studies.

2) Modulatory effects of PP corelease with norepinephrine will be studied physiologically in an isolated bath preparation of the vas deferens.

3) PP and CRF pathways in the rat brain will be determined by combining stereotaxic injections of retrogradely transported dyes and immunofluorescence.

Publications:

Jacobowitz, D.M. and Olschowka, J.A.: Coexistence of Bovine Pancreatic Polypeptide and Norepinephrine in the Central and Peripheral Nervous Systems. In Chan-Palay, V. and Palay, S. (Eds.): Coexistence of Neuroactive Substances New York, Wiley, 1983, in press.

Jacobowitz, D.M. and Olschowka, J.A.: Bovine pancreatic polypeptide-like immunoreactivity in brain and peripheral nervous system: Coexistence with catecholaminergic nerves. Peptides 3: 569-590, 1982.

Jacobowitz, D.M. and Olschowka, J.A.: Coexistence of bovine pancreatic polypeptide-like immunoreactivity and catecholamine in neurons of the ventral aminergic pathway of the rat brain. Brain Res. Bull. 9: 391-406, 1982.

Kobayashi, S., Olschowka, J.A. and Jacobowitz, D.M.: Bovine pancreatic polypeptide-like immunoreactive nerves in the rat major cerebral arteries. Brain Res. Bull. 10: 373-376, 1983.

Olschowka, J.A., O'Donohue, T.L., Mueller, G. and Jacobowitz, D.M.: Hypothalamic and extrahypothalamic distribution of CRF-like immunoreactive neurons in the rat brain. Neuroendocrinology 35: 305-308, 1982.

Olschowka, J.A., O'Donohue, T.L., Mueller, G. and Jacobowitz, D.M.: The distribution of corticotropin releasing factor-like immunoreactive neurons in the rat brain. Peptides 3: 995-1015, 1982.

Olschowka, J.A. and Jacobowitz, D.M.: The coexistence and release of bovine pancreatic polypeptide-like immunoreactivity from noradrenergic superior cervical ganglia neurons. Peptides 4 (2), 1983.

Ohhashi, T. and Jacobowitz, D.M.: The effects of pancreatic polypeptides and neuropeptide Y on the rat vas deferens. Peptides, in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00388-07 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Colocalization of Substance P and Acetylcholinesterase in Neurons of the Brain</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David M. Jacobowitz Chief, Histopharmacology Section LCS NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Immunocytochemical studies revealed the colocalization of substance P (SP) and acetylcholinesterase (AChE) within the <u>interpeduncular nucleus (ip)</u>. In the rat ip adjacent sections were stained for AChE and for SP-immunoreactivity. SP-fibers were found to be precisely colocalized with AChE-fibers in the dorsal cap and the lateral zones. Receptor autoradiography revealed that both <u>muscarinic receptors</u> (H^3-QNB ligand) and <u>α-bungarotoxin binding sites (nicotinic receptors)</u> had distributions resembling that of AChE. Neither unilateral nor bilateral lesions of the habenulae changed the number or distribution pattern of the receptors. The presence of SP immunoreactivity in areas of high nicotinic receptor densities provides a morphological basis for the previously suggested modulation of nicotinic responses by SP. The correlation between the localization of SP and AChE raises the possibility of a biologically relevant interaction between the two substances. Prior biochemical studies have provided evidence that AChE from several sources is capable of hydrolyzing SP. This evidence taken in conjunction with our demonstration of SP and AChE colocalization leads us to suggest that one physiological function of AChE in the ip may be to hydrolyze SP, thus terminating its biological activity.</p>		

Project Description:

Other Professional Personnel:

Andrej Rotter

Guest Worker

LCS NIMH

Objectives: Preliminary studies revealed a remarkable correlation between the localization of AChE staining nerves and substance P (SP) immunoreactive nerves within the interpeduncular nucleus (ip). In the present study, we have investigated in detail the distribution of AChE and SP neurons. We have also investigated the distribution of muscarinic acetylcholine receptors and α -bungarotoxin binding sites by autoradiography of receptor bound cholinergic ligands in order to determine their spatial relationship to AChE staining structures.

Methods Employed: 1) AChE histochemistry; 2) substance P immunocytochemistry; 3) receptor autoradiography for visualization of muscarinic and nicotinic receptors and 4) stereotaxic lesions.

Major Findings:

(A) Adjacent sections containing the ip of the rat brain revealed that SP immunoreactive fibers were precisely colocalized with AChE fibers in distinct regions of this structure. On the basis of AChE staining, the ip was subdivided into 5 zones. Intense AChE staining was observed in the dorsal cap and the lateral zones. Moderate staining was seen in the median zone and the dorsoventral column. The perivascular zones were unlabeled. SP and AChE staining was found colocalized in the lateral zones and the dorsal cap.

(B) Both muscarinic receptors and α -bungarotoxin binding sites had distributions resembling that of AChE. Neither unilateral nor bilateral lesions of the habenulae changed the number or distribution pattern of the receptors. It was concluded that cholinergic receptors are localized postsynaptically.

Significance to Biomedical Research and the Program of the Institute: We proposed that acetylcholine and SP-containing fibers terminate in well defined zones of the ip which contain nicotinic and muscarinic receptors and AChE. The presence of SP fibers in areas of high nicotinic receptor densities provides a morphological basis for the previously suggested modulation of nicotinic responses by SP. The correlation between the localization of SP and AChE raises the possibility of a biologically relevant interaction between the two substances. Recent biochemical studies (Chubb et al., 1980) have provided evidence that AChE is capable of hydrolyzing SP. This evidence taken in conjunction with our demonstration of SP and AChE colocalization leads us to suggest that one function of AChE in the ip may be to hydrolyze SP, thereby terminating its biological activity. These neuroanatomical studies lay the groundwork for a rational approach to studying the role of SP and AChE in the ip as a first step in revealing functional significance of this nucleus.

Proposed Course of the Project: Further studies on the interpeduncular nucleus are in progress.

Publications:

Jacobowitz, D.M. and Creed, G.J.: Cholinergic projection sites of the nucleus of the tractus diagonalis. Brain Research Bulletin 10: 365-371, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 00396-05 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Study of Proteins Within the CNS by Two-Dimensional Gel Electrophoresis		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) William E. Heydorn Guest Worker (NIGMS Fellow) LCS NIMH, NIGMS		
COOPERATING UNITS (if any) Laboratory of General and Comparative Biochemistry		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.7	PROFESSIONAL: 1.4	OTHER: 1.3
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Using <u>two-dimensional gel electrophoresis (2DE)</u>, we have constructed an atlas showing the <u>location</u>(on polyacrylamide gels) and <u>relative concentration</u> of a number of different proteins from 25 distinct neuroanatomical regions of the male rat brain. The regions examined included cortical areas as well as nuclei from the hypothalamus, amygdala, thalamus, forebrain and hindbrain. Proteins were separated by 2DE, <u>stained with silver</u> and <u>quantitated by computerized densitometry</u>. Some proteins were found to be present in constant amounts in all brain areas examined, others were found to vary somewhat in concentration and some (approximately 10%) varied widely (greater than 10-fold) in concentration among the different brain regions. Using this map, we have extended these observations by examining the effect of the tricyclic antidepressant <u>desmethylinipramine (DMI)</u> and the neurotransmitter depleting agent <u>reserpine</u> on proteins from the hippocampus and parietal cortex. Repeated administration of DMI caused a reduction in the apparent concentration of two proteins in both brain regions examined. In contrast, repeated reserpine administration caused an apparent increase in the concentration of both these proteins in the hippocampus, but no change in the parietal cortex. A third protein was elevated in concentration in the hippocampus by repeated DMI administration, and reduced in concentration by repeated reserpine administration. In all cases, acute drug administration was found to be without effect. This is significant in view of the fact that chronic DMI and reserpine have been shown to cause down and up regulation, respectively, of the β-adrenergic receptors. We will pursue the possibility that these proteins may be part of the catecholamine receptor.</p>		

Project Description:

Other Professional Personnel:

David M. Jacobowitz	Chief, Histopharmacology Section	LCS NIMH
David Goldman		LGCB NIMH
Carl R. Merrill		LGCB NIMH

Objectives: 1) Identify the molecular weight, isoelectric point and relative concentration of proteins in various regions of the male rat brain. 2) Investigate the effect of acute and chronic treatment of desmethylinipramine (DMI) and reserpine on the apparent concentration of proteins in various regions of the rat brain.

Methods Employed: 1) Two-dimensional polyacrylamide gel electrophoresis; 2) Photochemical silver staining of proteins on polyacrylamide gels; 3) Computerized scanning densitometry of proteins on two-dimensional gels; 4) Microdissection of discrete regions of the rat brain.

Major Findings:

(A) The results of our initial 2DE studies are the first attempt at systematically organizing the proteins visible on two-dimensional polyacrylamide gels from different neuroanatomical regions of the rat brain. We present the basis for a generalized system that will be used by us and others in future studies that deal with the classification of proteins from neuronal tissue.

(B) We have found that there exists marked differences in the apparent amount of individual proteins visible on two-dimensional polyacrylamide gels among various neuroanatomical regions of the rat brain. Of the proteins selected for densitometric examination, the majority (53%) varied less than 4-fold in concentration between the neuroanatomical areas with the lowest and highest detected amounts, although only 5% varied by less than 2-fold. In contrast, approximately 10% of the proteins examined varied widely in the quantity measured in each brain region, with concentration values ranging more than 10-fold between the regions with the lowest and highest detected amounts.

(C) Chronic administration of DMI produced a significant reduction in the apparent concentration of two proteins in both the parietal cortex and the hippocampus. One of these proteins has a molecular weight of 57,000 daltons and an isoelectric point (pI) of 6.2, while the second has the same molecular weight and pI 6.3. In contrast, a third, smaller protein (molecular weight 28,000 daltons, pI 5.9) was slightly elevated in both brain regions by chronic DMI administration, although this elevation reached statistical significance only in the hippocampus. In all cases, these proteins whose concentrations were altered by chronic drug administration were unaffected by acute drug treatment.

(D) In the hippocampus, the effect of repeated administration of reserpine was opposite to that detected after repeated treatment with DMI. Specifically, the two proteins of molecular weight 57,000 daltons whose apparent concentrations were reduced by repeated DMI administration were elevated in concentration after treating rats chronically with reserpine. The third protein which was elevated

by chronic DMI administration was reduced by repeated treatment with reserpine. In contrast, repeated administration of reserpine had no effect on these three proteins in the parietal cortex. Acute administration of reserpine also had no effect on any of the three proteins which were altered in concentration by repeated administration of the drug.

Significance to Biomedical Research and the Program of the Institute: The initial mapping studies of proteins within different neuroanatomical regions will form the groundwork for a single unitary system of classifying proteins visible on 2DE gels from neuronal tissue. This system will then be used by us and others when studying the effect of various experimental treatment on proteins within the CNS. Identification of a portion of a β -receptor protein in the brain will allow us to study the biochemical properties of the receptor.

Proposed Course of the Project:

- 1) Continuation of present studies with the goal of more clearly identifying proteins within different neuroanatomical regions of the rat brain that are affected by changes in noradrenergic neurotransmission and attempting to identify specific roles for these proteins in the maintenance of cellular homeostasis.
- 2) Using photoaffinity radioligands, identify the molecular weight and isoelectric point of various neurotransmitter receptor proteins in the central nervous system.
- 3) Proteins will be removed from the gels and injected into rabbits for the production of antibodies. Immunocytochemical studies will be used in an attempt to reveal the discrete localization of proteins of interest within the brain.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00397-05 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurophysiological Effects of Brain Peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Debra I. Diz Guest Worker (NIGMS Fellow) LCS NIMH, NIGMS		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">1.2</div>	PROFESSIONAL: <div style="text-align: center;">1.1</div>	OTHER: <div style="text-align: center;">0.1</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Our previous studies demonstrated that α-MSH, which has a widespread immuno-histochemical distribution in nerve fibers throughout the <u>hypothalamus</u>, increased <u>heart rate</u> (HR) when injected into the rostral dorsomedial <u>hypothalamic nucleus</u> (DM) but not in other sites in the hypothalamus. Studies on the <u>central cardio-vascular</u> (CV) effects of other neuropeptides suggest multiple sites of CV activity within the hypothalamus, which is in agreement with electrical stimulation data. For example, the potent <u>opiate receptor agonist</u>, <u>dermorphin</u> (40 pmol) produced increases in HR with minimal increases in <u>blood pressure</u> (BP) when injected into the anterior hypothalamic nucleus (AH). The response appears to be mediated through opiate receptors, and the mechanism involves activation of cardiac sympathetic nerves. <u>Thyroid releasing hormone</u> (TRH) also causes an increase in HR but not BP in the AH and the mechanism also appears to be activation of cardiac sympathetic nerves as indicated for dermorphin above. In addition, TRH (1.4 pmol) causes increases in BP and HR from sites in the preoptic supra-chiasmatic nucleus (POS), a response which appears to be due to a more general activation of the sympathetic nervous system and may include vagal withdrawal as a component of the increased HR. TRH has modest tachycardic effects in parts of the medial preoptic nucleus (POM) and in the DM and posterior hypothalamic nucleus (PH). <u>Bradykinin</u> resembles TRH in its site-dependent actions; increases in BP and HR in the DM and PH, tachycardia in the POS and POM and bradycardia in the PVN. Importantly, with each peptide, the CV responses were obtained in areas of the hypothalamus that have been reported to contain receptors and/or immuno-cytochemical evidence of fibers and cells containing the peptide. However, not all hypothalamic areas containing a given peptide responded with CV changes when the peptide was injected. General findings with all peptides tested were a relatively long duration of action (40 min to >90 min) and tachyphylaxis to repeated injections.</p> <div style="text-align: right;">(317)</div>		

Project Description:

Other Professional Personnel:

David M. Jacobowitz Chief, Histopharmacology Section LCS NIMH

Objectives: In an earlier study, we demonstrated that α -MSH, a peptide within dense terminal varicosities throughout the hypothalamus, produced moderate increases in heart rate and blood pressure when administered into the rostral dorsomedial nucleus. Injections into other hypothalamic areas were essentially without cardiovascular actions. The objectives of the present studies were 1) to determine the cardiovascular effects and discrete sites of action of other peptides known to be present within the hypothalamus (dermorphin, TRH, bradykinin); 2) to determine the characteristics of, and the mechanism responsible for, the cardiovascular actions; and 3) to determine if changes in respiration rate or rectal temperature accompany the cardiovascular responses to the peptides.

Methods Employed: 1) Stereotaxic injections of peptides using glass micropipettes; 2) measurement of arterial blood pressure and heart rate in anesthetized rats; 3) measurement of respiration rate and rectal temperature and 4) histological verification of injection sites in thionin stained, cryostat sectioned brain slices.

Major Findings:

(A) Dermorphin. 1) Identified specific caudal anterior hypothalamic (AH) sites in which 40 pmol dermorphin injections produced the greatest increases in heart rate (HR); smaller increases in HR occurred in the medial preoptic (POM) and paraventricular (PVN) nuclei and lateral ventricle, but not the dorsomedial (DM) or posterior (PH) hypothalamic nuclei. 2) Cardiovascular (CV) responses to dermorphin in the AH were blocked by intrahypothalamic naloxone, suggesting opiate receptors at the injection site are responsible for the CV actions of the peptides. 3) Pharmacological blockade of the vagus nerve and bilateral adrenalectomy prior to AH dermorphin failed to modify the response, but propranolol reversed or inhibited the response; therefore, activation of cardiac sympathetic nerves (rather than vagal withdrawal or adrenal catecholamine release) is responsible for the HR increase. 4) Neither respiratory rate nor rectal temperature were altered during the response to dermorphin.

(B) TRH. 1) Low dose TRH (1.4 pmol) increased HR and BP in the preoptic supra-chiasmatic (POS) and POM (7000-6800), decreased HR and BP in the POM (7400-7050) and increased HR only in the DM, PH and lateral ventricle. 2) Investigation of the mechanism of TRH-induced CV changes indicates that the increased HR in the AH is likely due to activation of cardiac sympathetic nerves. In the POS, vagal withdrawal and general sympathetic activation may both contribute to the increased HR and BP. 3) Respiratory rate and rectal temperature were not affected by low doses of TRH, larger doses increased respiratory rate.

(C) Bradykinin. 1) Initial work indicates decreases in HR occurred in the PVN, increases occurred in the POM and POS, and increases in both BP and HR occurred in the DM and PH; variable responses occurred in the AH depending on the rostro-caudal site of the injections. 2) Preliminary experiments with bradykinin

potentiating factor (a kininase II-inhibitor) suggests the compound has minimal CV actions after injections into the POS, POM or PVN and may increase BP and HR in the DM and PH.

Significance to Biomedical Research and the Program of the Institute: The recent demonstration by immunohistochemical methods of the presence of a variety of peptide substances within nerve cells and fibers in the central nervous system has led to the suggestion that these compounds play a role as neurotransmitters and/or neuromodulators. Few studies have been performed to elucidate the possible physiological actions of these neuropeptides. The present study of the cardiovascular actions of several brain peptides provides insight into one possible physiological brain action. The use of a discrete microinjection technique (versus widespread intracerebroventricular injections) provides additional information of specific hypothalamic areas responsible for the cardiovascular effects.

Proposed Course of the Project: Further studies to evaluate the mechanism of the bradykinin responses and the effects of local injections of inhibitors of the enzyme responsible for degrading bradykinin (kininase II) are in progress.

Publications:

Diz, D.I. and Jacobowitz, D.M.: Cardiovascular effects of intrahypothalamic injections of α -melanocyte stimulating hormone. Brain Research, in press, 1983.

O'Donohue, T.L.: Identification of endorphin acetylating enzyme (EAE) in rat brain and pituitary gland. Journal of Biological Chemistry 258(4): 2163-2167, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00400-01 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Protein Phosphorylation in Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Paul J. Marangos, Chief, Unit on Neurochemistry, Histopharmacology Sec., LCS, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The study of <u>protein phosphorylation</u> in brain is expected to provide in-formation relating to <u>receptor</u> linked effector mechanisms. During the past year, a new phenomenon has been observed in our laboratory. We have shown that the <u>brain specific protein, S-100</u>, is able to inhibit the phosphorylation of several synaptic proteins in a <u>calcium</u> dependent manner. The proteins characterized have molecular weights of 73, 56, 50 and 47 K. The 73 K protein is the most potently effected by S-100 and has been designated the S-100 <u>mediated phosphoprotein</u> or <u>SMP</u>. It is an acidic protein having an isoelectric point of 4.3, and efforts are underway to purify it from brain. The SMP is specific to brain and enriched in gray matter. The S-100 protein, therefore, appears to be a brain specific <u>calmodulin-type</u> protein with a reciprocal action to that of calmodulin. In other studies, an increased phosphorylation of a 45 K phosphoprotein has also been demonstrated in <u>amygdala kindled</u> animals. Phosphorylation of the 45 K protein is only increased in the amygdala of animals displaying <u>kindled seizures</u>. Animals exposed to ECS seizures or prekindled animals not displaying major motor seizures do not show increased phosphorylation of this protein. The 45 K protein may be involved in the behavioral sensitization process involved in kindling. </p>		

Project Description:

Other Professional Personnel:

Jitendra Patel	Visiting Fellow	LCS NIMH
Robert M. Post	Chief	BPB NIMH
David Jacobowitz	Chief, Histopharmacology	LCS NIMH

Objectives:

Our laboratory has for some time been involved in the study of drug and neurotransmitter receptors in brain. It is now evident that these membrane associated macromolecules constitute not only the initial recognition site for a specific neurotransmitter or neuromodulator but that they also represent an initial component of a complex transduction apparatus that triggers a biological response in the neuron. This response can result in the opening and closing of an ion channel for sodium, calcium or chloride, the synthesis of new proteins, the activation of various enzymes or any combination thereof.

The transduction of a receptor-ligand binding reaction into a biological response can probably occur by a variety of mechanisms, but to date only a few have been suggested. The most widely accepted of these involves the synthesis of a so called second messenger, such as cAMP. The increased cAMP levels activate protein kinases which phosphorylate specific proteins, thereby leading to functional alteration and a consequent biological response. Protein phosphorylation therefore plays a major role in various receptor mediated processes and can be considered a major mechanism of a variety of effector mechanisms.

In addition to cyclic AMP, Ca^{++} activated calmodulin has also been implicated as a modulator of protein kinase activity. Whereas, the phosphorylation of only several synaptosomal proteins has been shown to be modulated by cyclic AMP, many more proteins have been shown to be modulated by Ca -calmodulin. Voltage dependent increases in intracellular calcium levels may, therefore, lead to the activation of protein kinases and phosphorylation of many proteins. It is, therefore, likely that synaptic function is dependent on the state of phosphorylation of various proteins, which is in turn controlled by calcium-calmodulin mediated protein kinase activity.

Our study of synaptic protein phosphorylation is directed at a better understanding of synaptic efficacy at the molecular level. This system should prove valuable in further characterizing the molecular events which occur downstream from receptor-ligand interactions.

Methods Employed: 1) Polyacrylamide gel electrophoresis; 2) autoradiography and 3) kindling paradigm.

Major Findings:

Our initial efforts in this area focused on the effect of the benzodiazepines on synaptic membrane and soluble protein phosphorylation. We found that all benzodiazepines greatly inhibit Ca -calmodulin induced phosphorylation in a manner that is not reversed by specific benzodiazepine antagonists. This finding is of limited interest, since the lack of reversal by the antagonists indicates that the

effect of the benzodiazepines on synaptic protein phosphorylation is not receptor mediated.

During the past year, we have engaged in a series of studies concerning the effect of the brain specific protein, S-100, on protein phosphorylation. S-100 is a glial protein that was shown some years ago to specifically bind calcium. It is, therefore, essentially a brain specific calcium binding protein in search of a specific functional role. We have shown during the past year that the S-100 protein can modulate both soluble and membrane protein phosphorylation in brain. Our first study which has been published in *Biochem. Biophys. Res. Commun.* showed that the phosphorylation of four brain supernatant proteins having molecular weights of 73, 56, 50 and 47 K is inhibited by S-100. The 73 K protein is most sensitive to inhibition by S-100 with concentrations as low as 10 μ g/ml S-100 giving marked inhibition. This protein has been designated SMP or S-100 mediated phosphoprotein. This initial report suggested that the function of S-100 may be as a brain specific mediator of protein phosphorylation.

Further characterization of the effect of S-100 on brain protein phosphorylation has been done with the results showing that the inhibition of SMP phosphorylation by S-100 is calcium dependent. SMP is found in many brain areas, while being absent from peripheral tissues such as liver, kidney, pineal, pituitary and adrenal glands. The phosphorylation of SMP is inhibited by calmodulin, but to a lesser extent than by S-100. The inhibition of the phosphorylation of the 56, 50 and 47 K proteins occurs only at higher S-100 concentrations (20-50 μ g/ml) and the phosphorylation of these proteins is enhanced by calmodulin. These data are currently in press in the *Journal of Neurochemistry*. Very recent results show that SMP is also present in brain membranes and that there exists a variety of synaptic membrane proteins whose phosphorylation is inhibited by S-100. The isoelectric point of SMP has been shown by two dimensional gel electrophoresis to be 4.3, making it an acidic protein. Efforts are currently underway aimed at purifying SMP.

It therefore appears that the S-100 protein may function to modulate the phosphorylation of brain proteins in a manner analogous to that of calmodulin, although in a reciprocal fashion.

Studies are also in progress aimed at determining whether various behavioral, physiologic or pharmacologic treatments have effects on brain protein phosphorylation. Significant results have been obtained concerning the effects of electrical amygdala kindling on protein phosphorylation. The phenomenon of kindling is intriguing, since it represents a sensitization process which probably involves alterations in synaptic efficacy. The kindling process, therefore, represents a controlled manner of studying changes in synaptic function, a process which is quite relevant to understanding neural function. It has been shown that amygdala electrical kindling results in an increased phosphorylation of a 45 K membrane protein. The alteration is specific and seen at both the kindled foci (left amygdala) as well as in the right amygdala. Pre-kindled animals which do not display Stage 4 or 5 seizures do not display such an increase. The altered phosphorylation is apparently due to the kindled seizures and not the seizure itself, since animals given electroconvulsive seizures (ECS) do not display increases in 45 K protein phosphorylation. This rather specific effect implies that protein might be involved in the modulation of synaptic efficacy.

Significance to Biomedical Research and the Program of the Institute: The study of synaptic protein phosphorylation as induced by neurotransmitters, neuro-modulator drugs and behavioral manipulations can be expected to provided basic insights into neural function. This will therefore increase our understanding of the biological basis of behavior.

Proposed Course of the Project: These studies are expected to continue for the next several years.

Publications:

Patel, J., Marangos, P.J., Heydorn, W.E., Chang, G., Verma, A. and Jacobowitz, D.: S-100 mediated inhibition of brain protein phosphorylation. J. of Neurochemistry, in press.

Patel, J. and Marangos, P.J.: Modulation of brain protein phosphorylation by the S-100 protein. Biochem. Biophys. Res. Commun. 109: 1089-1093, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01831-07 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Basic and Clinical Studies of Neuronal and Glial Enolase		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Paul J. Marangos, Chief, Unit on Neurochemistry, Histopharmacology Sec., LCS, NIMH		
COOPERATING UNITS (if any) Royal Postgraduate Med. School, London; Duke Univ.: Laboratory of Neuropath. and Neuroanatomical Sciences, NINCDS; Oncol. Branch, NCI, NIMC, Bethesda, MD		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.5	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Studies involving neuron specific enolase (NSE) have during the past year focused on the clinical application of this new methodology. Studies on small cell lung cancer patients have continued with our previous serum data being validated. The diagnostic value of NSE in pediatric neuroblastoma patients has also been shown with 96% of Stage IV patients displaying highly elevated serum NSE levels. Serum NSE level is also predictive of survival time, especially in the infant to one-year-old patient group. We have shown that NSE radioimmunoassay and immunohistochemistry is able to identify virtually all neuroendocrine, APUD cells as well as tumors (APUDomas) derived from these cells. Basic studies have shown NSE to be a good index of neuronal regeneration with levels decreasing in brain areas that are innervated by damaged nerves. </p>		
(325)		

Project Description:

Other Professional Personnel:

J.M. Polak	Lecturer	Royal Med. Sch., London
A.G.E. Pearse	Professor	Royal Med. Sch., London
S. Bloom	Professor	Royal Med. Sch., London
D. Schmechel	Neurologist	Duke Univ.
John Minna	Chief, Medical Oncology Branch	NCI/NNMC
D. Carney	Oncologist	NCI/NNMC
A. Gazdar	Biochemist	NCI/NNMC
M. Brightman	Chief, Sec. on Neurocytology	LNNS/NINCDS

Objectives:

Our laboratory has had a long standing interest in proteins specific to the nervous system. The study of brain specific proteins is a viable approach towards determining the molecular mechanism of differentiated neuronal functions. Our efforts began some eight years ago when it was decided that the then recently isolated brain proteins termed 14-3-2 and S-100 presented interesting questions concerning their function. The 14-3-2 protein was thought to be neuronal and the S-100, glial. We have devoted substantial effort to both proteins and have since found that the S-100 protein appears to mediate protein phosphorylation in a manner opposite to that of calmodulin, while the 14-3-2 protein is a neuron specific form of the glycolytic enzyme enolase.

Our studies of the brain enolases have shown that three forms exist in this tissue. A dimer of two α subunits ($\alpha\alpha$), which we have called non-neuronal enolase (NNE), is analogous to liver enolase, and in nervous tissue, it is present only in glial cells. A dimer of two δ subunits ($\delta\delta$) is found only in neurons and has been named Neuron Specific Enolase or NSE. An intermediate hybrid form ($\alpha\delta$) is also found, which probably exists only during early development. Since the antisera to the α and δ subunit are totally distinct and show no cross reactivity, these two reagents have proven to be very useful, since the anti-NSE sera only stains neurons and the anti-NSE sera only glia. We, therefore, have specific probes for the two major cell types in brain.

Previous work from our lab has also shown that the appearance of the δ subunit (NSE) is strictly correlated to neuronal differentiation. Neurons only acquire NSE when they become functionally active and form synapses. Prior to this time, neurons contain the α subunit (NNE). Neuronal differentiation therefore involves a genetic switch from the α to the δ subunit. The respective antibodies therefore provide a powerful method of studying neuronal differentiation.

We have also shown that NSE is present in paraneuronal, neuroendocrine cells in the periphery. These cells have been termed APUD cells by Pearse, and through an extensive collaboration with him and Dr. Polak, we have shown during the past several years that NSE or the δ subunit is present in all APUD cells. This has offered an easy method of identifying these peptide secreting cells in the periphery. There is no other method for labeling virtually all APUD cells, and these cells are often quite sparsely distributed in various glands and organs, making the NSE methodology extremely valuable in this area.

Methods Employed: Radioimmunoassay, immunocytochemistry, clinical procedures -- such as diagnosis, blood drawing and surgery. Further basic and clinical studies of the system are the objectives.

Major Findings:

During the past year, we have focused quite heavily on clinical studies and the further delineation of NSE distribution in various neuroendocrine tissues. We have shown, in collaboration with Dr. Polak's group in London, a specific localization of NSE in the gut neuroendocrine APUD cells, studies that have been published in Gastroenterology and Experientia. The distribution of NSE in the endocrine system of the lung has also been determined with a hyperplasia of the endocrine cells demonstrated by NSE staining in asbestos treated animals. This study has been published in Histochemistry. Immunostaining with anti-NSE sera has also been shown to be a diagnostic tool for the identification of Merkel cell tumors of the skin. This study is currently in press in Cancer. NSE has also been shown to be present in the peptidergic innervation of the human male genital tract, studies which will be published in Investigative Urology. So NSE is a very useful tool for identifying the peripheral peptide secreting neuroendocrine cells and has attracted much attention among pathologists.

In collaboration with Dr. Prinz, we have also investigated serum neuron specific enolase in nonfunctioning islet cell (an APUD cell type) carcinoma. We have shown levels of NSE in these patients, although only several were studied. In one patient, the elevated serum NSE levels returned to normal after a radical pancreatectomy. These studies have been published in Lancet. We have looked at a variety of patients with neuroendocrine neoplasms and also found elevated serum NSE levels in some gastrinoma patients and insulinoma patients. This study was published in Surgery. In collaboration with Dr. Lloyd, we have also observed moderately elevated NSE serum levels in some medullary thyroid carcinoma patients, a finding that is currently in press in Cancer.

A major focus during the past year has revolved around small cell lung cancer, in collaboration with Dr. Minna's group. As we reported last year, almost 90% of small cell cancer patients with extensive disease have highly elevated serum levels of NSE. The levels decrease with tumor remission and increase upon relapse, making serum NSE levels potentially valuable as an index of the clinical course of the disease. This year, we have looked at non-small cell lung cancer patients who only rarely display elevated NSE levels. We have also looked at cerebrospinal fluid from small cell patients and have also seen dramatic elevations compared to normals. This data is currently being prepared for publication. We are also examining cultured small cells immunohistochemically to learn more about the distribution of NSE in these cells. The small cell data has generated much interest among clinicians in the small cell lung cancer area, and we are currently assaying sera from patients in several different clinics throughout the world.

During the past year, we have engaged in a major study of pediatric neuroblastoma patients in collaboration with Drs. Seeger and Zelter, both of whom are associated with the Children's Cancer Study Group at UCLA. These studies have proven to be very exciting, since we have shown that 96% of Stage IV neuroblastoma patients have highly elevated levels of serum NSE. In these studies, the serum NSE level was shown to be highly predictive of survival time, making

this methodology highly useful to clinicians. These studies are currently in press in Lancet. These studies are being continued with recent work showing that NSE immunostaining can detect neuroblastoma cells in the bone marrow of patients. This should prove to be highly useful as an aid to successful bone marrow transplants.

We have, therefore, during the past year shown that the NSE radioimmunoassay and immunocytochemical staining procedure has important clinical implications. We intend to further pursue this aspect of the project and have major collaborative efforts in the planning stages relating to Alzheimer's Disease. The NSE methodology is potentially useful in any disease which involves a pathology of either neurons or neuroendocrine cells.

In the basic research area during the past year, we have continued a collaborative project with Dr. Brightman, where we have shown that NSE can be used as an index of neuronal regeneration. In these studies, we showed that NSE immunoreactivity decreases markedly in the hypoglossal nucleus of brain, when the hypoglossal nerve is damaged. NSE levels return to normal upon regeneration of the nerve. A most interesting aspect of this study was that in the hypoglossal nucleus displaying decreased NSE levels, the neurons began to stain for non-neuronal enolase, indicating that neuronal degeneration involves a reverse switch to the nonspecific α subunit. These studies have been published in the Journal of Neuroscience.

We have recently begun a collaborative study with Dr. Brownstein directed at the ultimate cloning of the gene for NSE. This would be very informative, since we could then begin to ask questions -- such as -- What controls the synthesis of this major neuronal protein and what mechanisms are involved in mediating the switch from the α to the δ subunit that occurs coincident with neuronal differentiation? These studies will, therefore, hopefully provide basic insight into the biochemical mechanisms involved in neuronal differentiation, as well as provide an excellent model system for the study of brain protein synthesis.

Significance to Biomedical Research and the Program of the Institute: The NSE methodology has proven to be of broad importance to neurobiologists, since it represents the best method available for directly studying neurons and neuroendocrine cells. The recent clinical findings are exciting and indicate that the ability to measure the protein in patient material will be of major importance in small cell lung cancer and pediatric neuroblastoma cases.

Proposed Course of the Project: The ongoing basic, genetic and clinical studies will likely continue for several years.

Publications:

Bologa, L, Bisconte, J.C., Joubert, T., Marangos, P.J., Derbin, C., Rioux, F. and Herschkowitz, N.: Accelerated differentiation of oligodendrocytes in neuron-rich embryonic mouse brain cell cultures. Brain Res. 252: 129-136, 1982.

Prinz, R.A. and Marangos, P.J.: Serum neuron-specific enolase: A serum marker for non-functioning pancreatic islet cell carcinoma. Am. J. of Surgery 145: 77-81, 1983.

Lloyd, R.V., Sisson, J.C. and Marangos, P.J.: Calcitonin, carcinoembryonic antigen and neuron-specific enolase in medullary thyroid carcinoma: An immunohistochemical study. Cancer, in press.

Gu, J., Polak, J.M., Van Noorden, S., Pearse, A.G.R., Marangos, P.J. and Azzopardi, J.G.: Immunostaining of neuron enolase as a diagnostic tool for Merkel cell tumors. Cancer, in press.

Van Evercooren, A.B., Kleinman, H.K., Ohno, S., Marangos, P.J., Schwartz, J.P. and Dubois-Dalcq, M.E.: Nerve growth factor, laminin and fibronectin promote neurite growth in human foetal sensory ganglionic cultures. J. of Neuroscience Research, in press.

Kirino, T., Brightman, M.W., Oertel, W.H., Schmechel, D.E. and Marangos, P.J.: Neuron specific enolase as an index of reinnervation. J. of Neuroscience 3: 915-923, 1983.

Zelter, P.M., Marangos, P.J., Parma, A.M., Sather, H., Dalton, A., Siegel, S. and Seeger, R.C.: Elevated neuron-specific enolase in serum of children with metastatic neuroblastoma. Lancet, in press.

Ferri, G.L., Marangos, P.J., Bloom, S.R. and Polak, J.M.: Intramural distribution of neuron specific enolase (NSE) in the human gastrointestinal tract. Experientia, in press.

Sheppard, M.N., Kurian, S., Henzen-Logmans, S.C., Michetti, F., Cocchia, D., Cole, P., Rush, R.A., Marangos, P.J., Bloom, S.R. and Polak, J.M.: Neuron specific enolase and S-100: New markers for delineating the innervation of the respiratory tract in man and other mammals. Thorax, in press.

Whitehead, M.C., Marangos, P.J., Connolly, S.M. and Morest, D.K.: Synapse formation is related to the onset of neuron-specific enolase immunoreactivity in the avian auditory and vestibular system. Dev. Neurosci. 5: 298-307, 1982.

Campbell, I.C., Marangos, P.J., Parma, A.M., Garrick, N.A. and Murphy, D.L.: Localization of monoamine oxidases A and B in primate brain relative to neuronal enolases. Neurochemical Research 7: 657-666, 1982.

Sheppard, M.M., Johnson, N., Cole, G.A., Bloom, S.R., Marangos, P.J. and Polak, J.M.: Neuron specific enolase (NSE) immunostaining: A useful tool for the light microscopical detection of endocrine cell hyperplasia in adult rats exposed to asbestos. Histochemistry 74: 505-513, 1982.

Bishop, E., Polak, J.M., Facer, P., Ferri, G.L., Marangos, P.J. and Pearse, A.G.E.: Neuron specific enolase: A common marker for the endocrine cells and innervation of the gut and pancreas. Gastroenterology 83: 902-915, 1982.

Marangos, P.J.: Basic and Clinical Neurobiological Applications of Neuronal (NSE) and Non-neuronal (NNE) Enolase. In Scheuch, D.W., Haschen, R.J. and Hofmann, E. (Eds.): Multiple Forms of Enzymes. Berlin, Veb Verlag Volk Und Gesundheit, 1982, pp. 214-222.

Prinz, R.A. and Marangos, P.J.: Use of neuron-specific enolase as a serum marker for neuroendocrine neoplasms. Surgery 92: 887-889, 1982.

Vinores, S.A., Marangos, P.J. and Ko, L.: Butyrate induced increase in neuron specific enolase and ornithine decarboxylase in anaplastic glioma cell. Dev. Brain Res. 5: 23-28, 1982.

Vinores, S.A. and Marangos, P.J.: A developmental study of neuron specific enolase in rat adrenal medulla. J. Neurochem. 39: 1748-1751, 1982.

Zelter, P., Schneider, S., Bell, R. and Marangos, P.J.: Neuronal enolase and BB isoenzyme of creative kinase are biologic markers for neuroectodermal tumors. Clinical Res. 30: 139, 1982.

Marangos, P.J., Polak, J.M. and Pearse, A.G.E.: Neuron specific enolase: A probe for peptide secreting cells of the diffuse neuroendocrine system. 18th Symposium Medicum Hoechst. In Polak, J.M. and Bloom, S.R. (Eds.): Systematic Role of Regulatory Peptides. Stuttgart, F.K. Schattauer Verlag, 1983, pp. 447-465.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01833-03 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adenosine Receptors in the Central Nervous Systems		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Paul J. Marangos, Chief, Unit on Neurochemistry, Histopharmacology Sec., LCS, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH; Department of Physiology, University of Virginia; Neuroscience Branch, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20204		
TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.6	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> ³ Studies are in progress involving the <u>adenosine receptor</u> using the agonist [³H] <u>cyclohexyl-adenosine</u> and the antagonist [³H] <u>diphenylxanthine</u>. It has been shown that agonist and antagonist bind to either two distinct subtypes or conformations of the receptor, since the binding of these two ligands is differentially affected by several different treatments. Chronic <u>caffeine</u> treatment has been shown to cause a marked increase in the number of adenosine receptors in brain. The <u>adenosine uptake</u> inhibitor [³H] <u>nitrobenzylthioinosine</u> has been shown to be a photoaffinity probe for the adenosine uptake site with short exposures to ultraviolet light causing an apparently irreversible interaction. The sedative effects of adenosine have been shown to be potentiated by pretreatment of mice with nitrobenzylthioinosine. The relationship of adenosine receptors with calcium channels has prompted us to continue our study of the binding of the <u>calcium antagonist</u>, [³H] <u>nitrendipine</u> to brain membranes. The ontogeny of [³H] <u>nitrendipine</u> binding sites in chick brain and heart is being studied in an attempt to correlate the binding site to defined physiological processes. The study of ¹⁴C <u>2-deoxyglucose</u> uptake in brain and its regional modulation by adenosine agonists and antagonists is also under study in an effort to identify <u>functional adenosine receptors</u>. </p>		

Project Description:

Other Professional Personnel:

Jitendra Patel	Visiting Fellow	LCS NIMH
Jean-Phillipe Boulenger	Fogarty International Associate	BPB NIMH
Jean-Claude Bisserbe	Guest Worker	LCS NIMH
Robert M. Post	Chief	BPB NIMH
Nick Sperekalis	Professor of Physiology	U. Va.
Jacqueline N. Crawley	Staff Fellow	NSB NIMH

Objectives:

Adenosine is an extremely interesting purine that has over the past twenty years been shown to have a range of effects in both the CNS and the periphery. The antihypertensive effects of adenosine are expressed in its potent vasodilatory action and marked effect on blood pressure. The homeostatic control of blood pressure is probably a major function of adenosine in the periphery.

In the central nervous system adenosine has been shown to inhibit neuronal firing when applied iontophoretically. This effect is highly potent and is seen on very many of the tested cells. Adenosine also potently inhibits neurotransmitter release in many systems. To date, adenosine has been shown to inhibit the release of acetylcholine, norepinephrine, GABA, serotonin and dopamine. Preliminary studies indicate that the inhibitory effect of adenosine on calcium dependent release is due to the effect of adenosine on calcium influx. Behaviorally, adenosine is an extremely potent sedative with metabolically stable analogues exerting sedative effects at doses below 0.1 mg/kg. The methyxanthine caffeine is thought to exert its stimulant and anxiogenic effects via an antagonism of adenosine.

Biochemically, adenosine is an extremely potent modulator of cAMP levels. At low concentrations, adenosine causes decreases in cAMP levels, and at higher concentrations, increases in cAMP are observed. These effects have been designated A₁ and A₂, respectively. Adenosine has also been shown to potentiate the effects of other neurotransmitters, such as dopamine and norepinephrine, on cAMP levels.

All the effects of adenosine in both the CNS and the periphery are thought to be mediated by cell surface membrane receptors. It has, however, been difficult to study adenosine receptors directly, since membranes generate adenosine thru the action of the enzyme 5' nucleotidase. The recent development of metabolically stable adenosine analogues, such as cyclohexyladenosine (CHA) and diphenylxanthine (DPX), coupled with the incorporation of adenosine deaminase into the binding assay has made it possible to directly measure the adenosine receptor. The adenosine deaminase converts the endogenously generated adenosine to inactive inosine, while the [³H] CHA or [³H] DPX is not affected by adenosine deaminase. CHA is an adenosine agonist, and DPX is structurally related to caffeine and is an adenosine antagonist. The purpose of our studies in this area is to characterize the adenosine receptor system in brain and to develop new drugs active on this system.

Methods Employed: Receptor binding assays, autoradiography, 2-deoxyglucose methodology.

Major Findings:

Studies in our laboratory over the past several years have focused on the characterization of membrane associated adenosine receptors in an effort to gain insight into the mechanism of adenosine action in the CNS at the molecular level. We have also characterized the adenosine uptake site using as a probe the potent inhibitor of adenosine uptake [^3H] nitrobenzylthioinosine (NBI). Since it is likely that adenosine receptors are coupled in some manner to calcium channels due to the fact that adenosine is mainly active on calcium dependent processes, we are also studying voltage dependent calcium channels using the calcium antagonist [^3H] nitrendipine (NDP). The strategy is that characterization of these three systems, the adenosine receptor, the adenosine uptake site and the calcium channel, will provide insight into the relationship of these systems and provide a better understanding of adenosine actions.

Studies during the past year have focused on characterizing agonist and antagonist binding to the adenosine receptor. Major differences were found in the kinetics of binding, as well as other properties. [^3H] CHA binding is much more susceptible to proteolytic degradation and heat denaturation than is [^3H] DPX binding, suggesting that agonist and antagonist are binding to either different receptor subtypes or to different conformations of the same receptor. We have also further characterized the very interesting and specific effect of copper, zinc and cadmium on adenosine receptors. All metals are much more potent as inhibitors of agonist binding, and zinc and cadmium only inhibit 50% of antagonist binding. This provides additional support for multiple subtypes of the adenosine receptor. Furthermore, guanine nucleotides protected the [^3H] binding site from metal induced inactivation; whereas, they had no effect on metal induced inactivation of [^3H] DPX binding. This indicates that the agonist conformation of the receptor is linked to the regulatory GTP binding site. This is similar to other receptor systems that are linked to adenylate cyclase. These studies are in press in the Journal of Neurochemistry.

We have also shown in the past year that the anticonvulsant and antimanic drug carbamazepine is a quite potent and very specific inhibitor of adenosine antagonist binding. The K_i of carbamazepine inhibition of [^3H] DPX binding is $3\text{ }\mu\text{M}$ and for [^3H] CHA binding is $22\text{ }\mu\text{M}$. A dozen other neurotransmitter and neuro-modulator systems were tested in this study, of which only [^3H] diazepam and [^3H] RO-5-4864 were shown to be significantly affected by carbamazepine. A series of carbamazepine derivatives with varying anticonvulsant potencies were tested for their potency on the adenosine receptor. No significant correlation was observed between anticonvulsant efficacy and potency on the adenosine receptor, suggesting that the anticonvulsant actions of carbamazepine are probably not mediated by the adenosine receptor. It remains to be seen whether the antimanic properties of carbamazepine are due to its interaction with the CNS adenosine receptor. The results of this study are currently in press in the European Journal of Pharmacology.

A good deal of effort has been directed towards caffeine in the past year. Chronic caffeine administration studies have been done using caffeine supplemented diets. Diets containing 400 mg of caffeine/Kg of feed were used. This is equivalent in mice to a daily caffeine dose equivalent to 5-7 cups of coffee per day in humans. At 12, 26 and 40 days, the animals on chronic caffeine showed significant

increases in brain adenosine receptors compared to control animals. The increase was in the number of receptors, while the affinity remained unchanged. This study, therefore, demonstrates a classic antagonist effect for caffeine on brain adenosine receptors. The results of this study have been published in Life Sciences. Further studies have localized the caffeine induced increases to the cerebellum, brain stem and thalamus, while cerebral cortex and hippocampal binding sites are not significantly changed. Studies are also in progress to determine the effect of caffeine withdrawal and the effect of chronic caffeine on developing animals. These studies are providing the first real insight into the mechanism of caffeine's central actions.

Studies have also been done relating to the behavioral effect of the adenosine uptake inhibitor NBI on adenosine induced sedation. We have shown that NBI markedly potentiates the sedative effect of adenosine while having no effect on that of the metabolically stable analogues, such as CHA. This indicates that NBI is probably having a significant effect on synaptic concentrations of adenosine in vivo. The lack of effect of NBI on the sedative potency of the stable analogues supports this, since these compounds are not subject to the adenosine reuptake mechanism. These studies suggest that the adenosine reuptake blockers may be pharmacologically useful and are currently in press in Neuroscience Letters.

We have shown during the past year that [^3H] nitrobenzylthioinosine is a photoaffinity probe for the adenosine uptake site. Exposure of membranes with [^3H] NBI bound to them to ultraviolet light leads to an apparently irreversible interaction, since excess unlabeled ligand is unable to displace the photoactivated [^3H] NBI. The degree of irreversibility of the photolabeling process has yet to be determined, but efforts are currently underway to visualize the binding site on SDS gels. This study has been published in the European Journal of Pharmacology.

We have for the past year also been engaged in a major study aimed at determining the developmental profile of the voltage dependent calcium channel in both brain and heart of the chick. We are in this study utilizing [^3H] nitrendipine binding as our index to determine the number of calcium channels. The chick has been chosen for this study, since extensive electrophysiology has been performed, and the binding results can be directly related to the appearance of calcium conductance data. To date, we have shown that the calcium antagonist binding site is fairly high in the embryonic chick heart, while it appears late in chick brain. These studies have been performed in collaboration with Dr. Sperekalis at the University of Virginia.

We have also during the past year set up in our own laboratory the procedure to do the 2-deoxyglucose uptake protocol. Since we have mapped the location of brain adenosine receptors, it now becomes important to see which areas of brain display altered 2-deoxyglucose uptake in response to adenosine agonist administration. We hope in this manner to identify functional adenosine receptors in brain. These studies are being done by Dr. Bisserbe, a guest worker from France.

Significance to Biomedical Research and the Program of the Institute: The adenosine system is likely to represent a major effector of behavior as relates to the mediation of sedation and anxiety. The potential for new drug development relating to neurologic and psychiatric disorders is high and virtually untapped for this system. Basic information relating to the biochemical basis of

behavior and especially hypertension should also result from an increased understanding of the adenosine systems.

Proposed Course of the Project: The scope of our studies has expanded during the past year, and we expect this project to continue for at least the next two years.

Publications:

Marangos, P.J., Patel, J., Martino, A.M., Dilli, M. and Boulenger, J-P.: Differential binding properties of adenosine receptor agonists and antagonists in brain. J. of Neurochemistry, in press.

Boulenger, J-P, Patel, J., Post, R.M., Parma, A. and Marangos, P.J.: Effets de l'administration prolongee de cafeine sur les recepteurs cerebraux de l'adenosine et des bensodiazepines. Actual. Pharm. Biol. Clin., in press.

Crawley, J.N., Patel, J. and Marangos, P.J.: Adenosine uptake inhibitors potentiate the sedative effects of adenosine. Neuroscience Letters, in press.

Marangos, P.J., Patel, J., Skolnick, P. and Paul, S.M.: Endogenous "Benzodiazepine-like" Agents. In Usdin, E., Skolnick, P., Tallman, J.F., Greenblatt, D. and Paul, S.M. (Eds.): Pharmacology of the Benzodiazepines. New York, Macmillan Press, 1983, pp. 519-529.

Boulenger, J-P, Patel, J., Post, R.M., Parma, A.M. and Marangos, P.J.: Chronic caffeine consumption increases the number of brain adenosine receptors. Life Sci. 32: 1135-1142, 1983.

Marangos, P.J., Clark-Rosenberg, R. and Patel, J.: [³H] nitrobenzylthioinosine is a photoaffinity probe for adenosine uptake sites in brain. Europ. J. of Pharmacology 85: 359-360, 1982.

Marangos, P.J., Patel, J., Miller, C. and Martino, A.M.: Specific calcium antagonist binding sites in brain. Life Sci. 31: 1575-1585, 1982.

Marangos, P.J., Post, R.M., Patel, J., Zander, K., Parma, A. and Weiss, S.: Specific and potent interactions of carbamazepine with brain adenosine receptors. Eur. J. of Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01834-06 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Endogenous Ligands for the Brain Benzodiazepine Receptor		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Paul J. Marangos Chief, Unit of Neurochemistry, Histopharmacology Sec., LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIAMDD; Biological Psychiatry Branch, NIMH; E.I. Dupont Company, Glenolden, Pennsylvania; Psychiatrische Universitäts Klinik, Basel, Switzerland		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Histopharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) TERMINATED. Combined with Z01 MH 01833-03 LCS.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00401-18 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Peripheral Noradrenergic Function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) Laboratory of Bioorganic Chemistry, NIADD, NIH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Medicine		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 3.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The objectives of this project are to elucidate biochemical mechanisms controlling the <u>synthesis, storage, release, action and termination of action of norepinephrine</u> in the adrenergic neurones and how to assess these in the intact animal. This year attention has focused <u>6-fluoronorepinephrine</u> as a substitute transmitter for norepinephrine and on evaluation of functional aspects of α -adrenoceptor function, particularly relating <u>intrajunctional (α_1-) and extra-junctional (α_2-) receptors</u> to alterations of responses to sympathetic stimulation or administered norepinephrine after inhibition on transmitter uptake by sympathetic neurones. The role of the sympathetics in the effect of PCP were examined also.		

Other Professional Personnel Engaged on Project:

Kenneth L. Kirk	Research Chemist	NIAAD NIH
David C. Jimerson	Staff Psychiatrist	LCS NIMH
David Lozovsky	Guest Worker	LCS NIMH
Virginia K. Weise	Chemist	LCS NIMH
Isamu Yamaguchi	Guest Worker	LCS NIMH
Giora Feuerstein	Guest Worker	LCS NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed Bayorh	Visiting Fellow	LCS NIMH

Objectives: To evaluate neurotransmitter function in animals and patients it is important for the understanding of brain function to determine the sites and rates of utilization of the substance involved in transmission of impulses at synapses in the brain and ganglia and at peripheral neuroeffector junctions.

Methods Employed: Pithed rats are used to study effects of sympathetic stimulation on norepinephrine infusion on blood pressure, heart rate, and plasma catecholamine levels. The effects on these of various drugs and hormones are examined. Blood flow and cardiac output are measured with cobalt-labelled microspheres. A potential means for examining neurotransmitter function in intact humans involves the use of ^{18}F -labelled compounds which can act as (or be converted to) substances which are selectively taken up and retained at the same sites as a normal endogenously formed neurotransmitter combined with position emission tomography (PET) scanning.

Major Findings: After developing techniques for separation and assay of fluorinated catecholamines (Drs. Chiueh, Daly and Kopin) demonstrated 6-fluoro-dopamine (6-F-DA) was to be selectively taken up into peripheral sympathetic neurones and converted to 6-fluoro-norepinephrine and is released by sympathetic nerve stimulation. Furthermore, the turnover rate of 6-F-NE is the same as that of ^3H -NE, which indicates that the fluorinated derivative can be considered a valid tracer molecule for endogenous norepinephrine. Plans are in progress for the production and use of 6- ^{18}F -DA for use in study of peripheral sympathetic function. Studies with 6-fluoro-DOPA are also being considered to establish its usefulness in studying brain catecholamines.

Previously reported studies from this laboratory have shown that after administration of drugs which selectively block α_1 -adrenoceptors, the pressor responses to stimulation of sympathetic outflow from the spinal cord of pithed rats are more effectively inhibited than are responses to administered NE. After α_2 -adrenoceptor blocking agents, the pressor responses to administered NE are inhibited more effectively than responses to stimulation. On the basis of these observations, it was proposed that α_1 -adrenoceptors are mainly intrajunctional whereas α_2 -adrenoceptors are located at sites outside the neuroeffector junction.

Effects of α_1 and α_2 -adrenoceptor blocking agents on the pressor response to sympathetic stimulation are additive, indicating that administered norepinephrine reaches both types of receptors. Desmethylinipramine and cocaine, which block reuptake of norepinephrine into sympathetic nerves, do not greatly potentiate the pressor response to stimulation of the sympathetic outflow from the spinal cord of pithed rats, but do prolong and potentiate the effects of administered norepinephrine. This difference is in part due to the effects of presynaptic

α_2 -adrenoceptors which modulate feedback control by norepinephrine of its own release. When uptake is inhibited a greater fraction of the released norepinephrine reaches extrajunctional (α_2) adrenoceptors, decreases the amounts of norepinephrine released so that the net action of α_1 -adrenoceptors in the junction and the overflow of norepinephrine to α_2 -adrenoceptors is not greatly altered. This interpretation is supported by the effects of yohimbine (an α_2 -adrenoceptor blocking agent) and prazosin (α_1 -adrenoceptors blocking agent) in pithed rats pretreated with DMI. Inhibition of uptake by DMI reverses more effectively the inhibition by yohimbine than by prazosin of stimulation-induced pressor responses. This attributed to the reversal of the presynaptically (α_2 -adrenoceptor) mediated inhibition of norepinephrine release (Drs. Zukowska-Grojec, Bayorh and Kopin).

The effects of inhibition of uptake by DMI on the pressor effects of administered norepinephrine after treatment of pithed rats with yohimbine or prazosin also support the view that α_2 -adrenoceptors are extrajunctional and α_1 -adrenoceptors are intrajunctional. After inhibition of α_2 -adrenoceptors (yohimbine), DMI potentiates by 10-fold pressor effects of administered norepinephrine at the remaining α_1 -receptors. This is due to a combination of higher plasma levels of norepinephrine (because uptake is inhibited everywhere) and greater accessibility of plasma NE to the α_1 -(intrajunctional) receptors. Potentiation by DMI of the pressor responses after inhibition of α_1 -adrenoceptors is only about 3.5-fold. Since the remaining (α_2 -) receptors are extrajunctional, the potentiation is mainly a result of higher plasma NE levels. The difference in potentiation by DMI of actions of administered NE at α_1 -(intrajunctional) and α_2 -(extrajunctional) receptors may be explained by their location in relation to the site of NE uptake which is inhibited by DMI.

Tyramine is an indirectly sympathomimetic agent which owes its effects to releasing norepinephrine from sympathetic nerves. Tyramine is chemically related to norepinephrine and also competes for uptake sites on the sympathetic axon. In adrenal demedullated rats, the relationship between increased plasma NE and pressor responses during administration of tyramine is similar to that seen during sympathetic stimulation in DMI pretreated pithed rats. These results suggest that the effects of tyramine on blood pressure appear to be mediated by norepinephrine release at sites similar to that evoked by nerve stimulation. The increment in plasma norepinephrine is greater because after either tyramine or DMI, norepinephrine uptake into the sympathetic nerves is blocked and a greater fraction of the released transmitter reaches the circulation (Drs. Bayorh, Zukowska-Grojec and Kopin).

In pithed, adrenalectomized rats treated with sufficient yohimbine to block α_2 -adrenoceptors, during sympathetic stimulation there is a direct linear relationship between the measurement in blood pressure and the logarithm of the increment in plasma norepinephrine. Similarly, during infusion of norepinephrine, the increment in blood pressure parallels the logarithm of the increment in plasma norepinephrine, but the norepinephrine levels attained during infusion must be about 10-fold higher than during stimulation to attain the same blood pressure. Treatment with DMI shifts the linear relationships between plasma norepinephrine and blood pressure by about three-fold, but in opposite directions so that during infusion lower levels of norepinephrine are required to attain the same pressor response, whereas during stimulation, a given pressor response is associated with three-fold higher plasma norepinephrine levels. The interpretation of these

results leads to the conclusion that norepinephrine levels at the neuroeffector junction are the geometric mean of those found in plasma during stimulation and norepinephrine infusion.

During continuous sympathetic stimulation in pithed rats, the evoked pressor response slowly declines. Cobalt-57 labelled microspheres were used to measure cardiac output, organ blood flow and vascular resistance during stimulation-induced responses in demedullated, yohimbine-pretreated rats and contrasted with effects seen with similar blood pressure increases during continuous 1-norepinephrine infusion. The decline of MPB during constant stimulation results from a decrease in cardiac output. Opposite effects of stimulation and norepinephrine on regional blood flow and vascular resistance may indicate differences in accessibility of circulating vs. endogenously released norepinephrine to adreno-receptors in various vascular beds (Drs. Zukowska-Grojec, Bayorh and Kopin).

Phencyclidine (PCP) is a frequently abused drug which evokes marked sympathetic responses. By examining the effects of the drug on blood pressure, heart rate and plasma catecholamine responses in intact animals and in pithed rats, it is possible to separate actions involving peripheral sympathetic nerves and those involving the central sympathetic regulatory mechanisms. PCP was shown to produce a four-fold increase in both epinephrine and norepinephrine levels in plasma of intact rats (Drs. Bayorh and Kopin). In pithed rats, PCP increased the plasma norepinephrine levels less than two-fold, indicating the PCP both evokes a centrally mediative enlargement of sympatho-adrenal medullary activity and increases plasma catecholamines by an action in the peripheral tissue. Since it was shown that PCP does not present uptake by ^3H -NE by sympathetic nerves nor prolong the action of administered norepinephrine, it can be concluded that the drug enhances release of the catecholamine (Drs. Bayorh and Kopin).

Significance to Biomedical Research and to the Program of the Institute:

Norepinephrine is the neurotransmitter released for sympathetic nerves and some neurones in brain. Understanding its formation, disposition, metabolism and action are fundamental to defining its rate in disease states and during drug action.

Proposed Course:

Continued study of processes and their regulation in experimental animals.

Publications:

Kopin, I.J.: The evolving views of the metabolic fate of norepinephrine. *Endocrinologia Experimentalis* 16: 291-300, 1982.

Chieh, C.C., Zukowska-Grojec, Z., Kirk, K.L. and Kopin, I.J.: 6-Fluoro-catecholamines as false adrenergic neurotransmitters. *J. Pharmacol. Exp.-Ther.* (in press).

Bayorh, M.A. and Kopin, I.J.: Effect of phencyclidine (PCP) on adrenergic responses to spinal cord stimulation in pithed rats. *Eur. J. Pharmacol.* 85: 15-21, 1982.

Bayorh, M.A., Zukowska-Grojec, Z. and Kopin, I.J.: Effect of desipramine and cocaine on plasma norepinephrine and pressor responses to adrenergic stimulation in pithed rats. J. Clin. Pharmacol. 23: 24-31, 1983.

Zukowska-Grojec, Z., Bayorh, M.A. and Kopin, I.J.: Effect of desipramine on the effects of α -adrenoceptor inhibitors on pressor responses and release of norepinephrine into plasma of pithed rats. J. Cardiovasc. Pharmacol. 5: 297-301, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00402-11 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) CNS Regulation of Autonomic and Endocrine Function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) Sections on Histopharmacology and Pharmacology Department of Neurobiology, USUHS Hadassah Hospital, Jerusalem, Israel		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Medicine		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 2.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> The purpose of this project is to define the regions of the brain, the neuronal pathways, and the neurotransmitter systems which control endocrine, metabolic and autonomic responses to internal stimuli and to various types of stress and the effects of drugs on these systems. Studies on intracerebral injections of prostaglandin $F_{2\alpha}$ and opiates were completed. Changes in dopamine receptors were examined in relation to the role of prolactin in producing super-sensitivity of dopamine receptors. Vasopressin mediation of baroreflexes in the brain and its role in sustaining blood pressure responses to baroreceptor deafferentation were examined. </p>		

Other Professional Personnel Engaged on Project:

Alan I. Faden	Chief, Neurobiology Research Unit	USUHS
Charles Saller	Guest Worker	LCS NIMH
David Lozovsky	Guest Worker	LCS NIMH
Giora Feuerstein	Guest Worker	LCS NIMH
Andreas Pfeiffer	Guest Worker	LCS NIMH
Chuang Chiueh	Sr. Staff Fellow	LCS NIMH
Miklos Palkovits	Visiting Scientist	LCS NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed Bayorh	Visiting Fellow	LCS NIMH
Robert Zerbe	Sr. Staff Fellow	LCS NIMH
Drori Ben-Ishay	Associate Prof. Medicine	Hadassah Hosp. Israel

Objectives: To determine the mechanisms involved in the regulation by the central nervous system of respiratory, metabolic, endocrine and cardiovascular function.

Methods Employed: Surgical techniques for lesioning or microinjection of appropriate pharmacological agents and cerebral ventricles or specific areas of brain, blood pressure, heart rate and changes in plasma catecholamines monitored. Microdissection and microassay of putative neurotransmitters aid identification of specific neuronal systems.

Major Findings: A series of studies on the cardiovascular and metabolic effects of prostaglandins injected directly into the cerebral ventricles were completed. $\text{PGF}_{2\alpha}$ injected into the cerebral ventricles elicits increases in heart rate, blood pressure, respiration and rectal temperature. Vagotomy has little effect on the responses, but hexamethonium blocks the pressor and temperature responses but not the heart rate or respiratory responses. Removal of the kidneys abolishes the residual respiratory, pressor, and temperature responses in hexamethonium-treated rats, indicating that renin-angiotensin might be involved (Drs. Feuerstein, Helke, Jacobowitz, Zerbe and Kopin).

The role of opiate peptides in the regulation of cardiovascular responses to hypotension and control of blood pressure were continued. Intracerebroventricular (icv.) injections of selective opioid agonists were utilized to investigate the role of opiate receptor subtypes in cardiovascular function in awake rats. The μ -agonist (D-Ala², MePhe⁴, Gly⁵-O¹) enkephalin (DAGO) (1 nmol) caused a prolonged increase in blood pressure and an initial decrease followed by a delayed increase in heart rate. These effects were antagonized by the selective μ -antagonist β -flunaltrexamine (β -FNA). A selective δ -agonist (dimeric tetrapeptide enkephalin) (DTE₁₂) was devoid of cardiovascular effects at 10 nmol, while a κ -agonist (MRZ) caused a pressor response which was not antagonized by β -FNA (Drs. Pfeiffer, Feuerstein, Zerbe, Faden and Kopin).

The mechanisms by which opioids elicit cardiovascular effects were analyzed in detail using microinjections into the anterior hypothalamic area. Low doses of enkephalin produced increases in heart rate and blood pressure. Associate elevations of plasma norepinephrine (NE) and epinephrine (EPI), but not vasopressin, suggested a stimulation of sympatho-adrenomedullary pathways. Higher doses caused increases in blood pressure but decreases in heart rate. Peripheral vagal blockade with atropine methyl nitrate (ATMN) caused a large sudden rise in heart rate indicating that an increased vagal outflow counteracted the sympathetic

activation. Adrenal demedullated rats displayed no tachycardia after anterior hypothalamic injection of low doses of enkephalin, while high doses caused pronounced bradycardia. Additional treatment of demedullated rats with the sympathetic blocker bretylium led to severe hypotension in addition to bradycardia. These data provide evidence that μ -opiate receptors mediate primarily cardiovascular effects of opiates in awake rats. At low doses, a sympathetic adrenomedullary activation occurs, whereas higher doses additionally activate parasympathetic efferents both possibly from anterior hypothalamic sites (Drs. Pfeiffer, Feuerstein, Zerbe, Faden and Kopin).

Chronic treatment with Domperidone, a dopamine receptor blocking agent which does not penetrate to the brain, produces dopamine receptor supersensitivity in the corpus striatum, indicated by enhanced binding of ^3H -spiroperidol and behavioral effects of apomorphine (Drs. Jimerson, Saller, Lozovsky and Kopin). This is believed to be an indirect effect, secondary to drug-induced hyperprolactinemia.

The acute hypertension and tachycardia which is produced in rats by bilateral section of the nerve fibers entering the Nucleus Tractus Solitarius is accompanied by marked increases in plasma norepinephrine, epinephrine and vasopressin. Similar effects were found in chlorisondamine-treated normal rats and in Brattleboro rats (which cannot form vasopressin). Treatment of Brattleboro rats with chlorisondamine abolished completely the increases in blood pressure and heart rate, indicating that either sympathetic discharge of vasopressin are sufficient to maintain the pressor response to baroreceptor deafferentation, but at least one of these is essential to the response (Drs. Zukowska-Grojec, Bayorh, Zerbe, Palkovits and Kopin).

Vasopressin and catecholamines are present in the Nucleus Tractus Solitarius of rats. In hypertensive rats of the SABRA strain, levels of norepinephrine, epinephrine and vasopressin are higher than in normotensive rats, suggesting that these pressor agents are involved in the response to, or pathogenesis of, this genetic form of hypertension (Drs. Feuerstein, Zerbe, Ben-Ishay, Kopin and Jacobowitz).

Significance to Biomedical Research and the Program of the Institute:

The mechanisms involved in the regulation of essential bodily functions to maintain homeostasis or to respond to stress are the basis of survival. Understanding these processes and how they are modified in disease states or by drugs is essential for rational therapy of a wide variety of neuropsychiatric disorders.

Proposed Course:

Continued studies on areas of brain required for metabolic effects, cardiovascular responses, etc. and evaluation of the roles of various peptides and other neurotransmitters, and the effects of drugs which affect neurotransmitter function.

Publications:

- Feuerstein, G., Helke, C., Zerbe, R.L., Jacobowitz, D.M. and Kopin, I.J.: Mechanisms involved in central cardiovascular effects of prostaglandin $F_{2\alpha}$. Am. J. Physiol. 242: R454-R551, 1982.
- Feuerstein, G., Zerbe, R.L., Ben-Ishay, D., Kopin, I.J. and Jacobowitz, D.M.: Catecholamines and vasopressin in hindbrain nuclei of hypertension prone and resistant rats. Brain Res. 251: 169-173, 1983.
- Zerbe, R.L., Bayorh, M.A., Quirion, R., and Kopin, I.J.: The role of vasopressin suppression in phencyclidine-induced diuresis. Pharmacology 26: 73-78, 1983.
- Zukowska-Grojec, Z., Bayorh, M.A., Kopin, I.J. and Feuerstein, G.: Leukotriene D_4 : Cardiovascular and sympathetic effects in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. J. Pharmacol. Exp. Ther. 223: 183-189, 1982.
- Zukowska-Grojec, Z., Bayorh, M.A., Zerbe, R.L., Palkovits, M. and Kopin, I.J.: The role of catecholamines and vasopressin in cardiovascular responses to deafferentation of the Nucleus Tractus Solitarius in the rat. Hypertension (in press) 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00403-10 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Indices of Adrenergic Function in Humans		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS, NIMH Hypertension - Endocrine Branch, NHLBI		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Medicine		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The levels of <u>norepinephrine</u>, <u>epinephrine</u>, and <u>dopamine</u> and their metabolites in various body fluids reflect the activity of the neurones from which these neurotransmitters are released. Urinary <u>catecholamine metabolites</u> provide an index of overall synthesis in the body while the relative proportion of nor-metanephrine appears to be related to the functional activity. Although plasma levels of norepinephrine reflect the responses of the peripheral <u>sympathetic nervous system</u> it is necessary to consider removal rates of the catecholamine. <u>Cerebrospinal fluid</u> levels of catecholamine metabolites can be used to assess central nervous system metabolism if appropriate corrections are made for the contribution from plasma. A more thorough understanding of neurotransmitter metabolism leads to more rational approaches to therapy. </p>		

Other Professional Personnel Engaged on Project:

Ronald J. Polinsky	Medical Officer (Neurology)	LCS NIMH
David Goldstein	Senior Staff Fellow	NHLBI NIH
Robert T. Brown	Medical Staff Fellow	LCS NIMH
Michael H. Ebert	Chief, Section on Experimental Therapeutics	LCS NIMH

Objectives: To develop methods for assessing adrenergic activity in the brain and peripheral sympatho-adrenal system using assays of catecholamines and their metabolites in body fluids and to apply these methods to the study of disease states and mode of action of therapeutic agents.

Methods Employed: Levels of endogenous catecholamines and their metabolites in cerebrospinal fluid, blood and urine are measured under basal conditions and after evoking a sympathetic response. Isotopically labelled catecholamines or their metabolites are infused intravenously and their levels in the various body fluids examined and related to kinetic parameters. Analyses are performed using liquid scintillation spectrometry, high pressure liquid chromatography, and mass spectroscopy.

Major Findings: Patients with orthostatic hypotension due to brain disease (Multiple System Atrophy, MSA) excrete abnormally low amounts of normetanephrine, but almost normal quantities of the deaminated major metabolites, 3-methoxy,4-hydroxyphenyl glycol (MHPG) and 3-methoxy,4-hydroxymandelic acid (vanillylmandelic acid, VMA). Patients with Idiopathic Orthostatic Hypotension (IOH) also secreted markedly diminished amounts of normetanephrine, but the excretion of VMA and MHPG is also depressed. The disproportionate decrease in normetanephrine in patients with MSA is attributed to a lack of stimulation of a relatively intact peripheral sympathetic nervous system. In IOH, all metabolites are diminished because of involvement of the peripheral sympathetic nerves resulting in a proportional decrease in all metabolites.

Infused MHPG labelled with deuterium was used to measure the rates of formation and utilization of free MHPG in plasma. Free MHPG accounted for about two-thirds of the total norepinephrine metabolites in urine, indicating it is the major intermediate in norepinephrine metabolism.

When a mixture of ^3H -L- and ^{14}C -D-norepinephrine is infused with ^3H -isoproterenol, there is a slower decline of plasma ^3H -isoproterenol than of the D- or L-norepinephrine reflecting a lack of uptake of isoproterenol into the sympathetic neurones. Desipramine, an antidepressant which inhibits norepinephrine uptake, slows the decline in plasma levels of the ^3H and ^{14}C labelled norepinephrine to the rate of decline in ^3H isoproterenol which is unaffected by the drug. Similarly in patients with IOH, but not in those with MSA, the rate of decline of labelled norepinephrine in plasma resembles that of isoproterenol, consistent with absence of sympathetic neurones.

MHPG in CSF and in plasma are lowered in both IOH and MSA. Normally between 30 and 50 percent of MHPG in CSF is derived from plasma and the remainder from the central nervous system. In patients with MSA, the proportion of CSF MHPG derived from the central nervous system is diminished, whereas in patients with IOH, the proportion of CSF MHPG derived from peripheral norepinephrine is diminished. This is consistent with a central nervous system deficit in MSA

and peripheral sympathetic involvement in IOH.

A method for adjustment of blood pressure by automatic feedback-regulated intravenous infusion from a computer controlled pump of norepinephrine has been developed and its use in patients with orthostatic hypotension successfully tested over an interval of several hours. The device is being improved to allow longer intervals of infusion of the pressor agent. This "sympathetic prosthesis" is useful in any form of hypotension.

Significance to Biomedical Research and to the Program of the Institute:

Assessment of adrenergic function by biochemical measurements provides a diagnostic tool and provides the insights into mechanisms of neurological and psychiatric disorders which are necessary to develop rational approaches to therapy.

Proposed Course: Apply the techniques developed to study of drug effects, endocrine-induced changes, consequences of stress, etc. Improve the sympathetic prosthesis and examine consequences of long-term norepinephrine infusion on receptor function.

Publications:

Kopin, I.J., Polinsky, R.J., Oliver, J.A., Oddershede, I.R. and Ebert, M.H.: Urinary catecholamine metabolites as indices of sympathetic neuronal dysfunction in patients with orthostatic hypotension. J. Clin. Endocrinol. Metab. In press.

Kopin, I.J.: Catecholamines and the cardiovascular system - recent advances. Plenary lecture 5th International Catecholamine Symposium, Goteborg, Sweden June 1983 (In press).

Kopin, I.J.: Avenues of investigation for the role of catecholamines in anxiety. VII World Congress of Psychiatry, Vienna, Austria, July 1983 (In press).

Polinsky, R.J., Samaras, G.M. and Kopin, I.J.: Sympathetic neural prosthesis for managing orthostatic hypotension. Lancet, 901-904, 1983.

Polinsky, R.J., Goldstein, D., Horwitz, D., Keiser, H. and Kopin, I.J.: Plasma catecholamine kinetics in patients with chronic autonomic failure and control subjects. 5th International Catecholamine Symposium, Goteborg, Sweden, June 1983 (In press).

Bybee, D.E., Wiesen, M., Aronin, N., Krieger, D.T., Frohman, L.A. and Kopin, I.J.: Failure of bromocriptine to lower plasma catecholamines in normal men and women. J. Clin. Endocrinol. Metab. 54: 648-652, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00404-12 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunological Localization of GAD and CSD in Neurones and Other Cells		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) State University of Connecticut, Storrs, Connecticut		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Medicine		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0	PROFESSIONAL: 0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;">This project has been discontinued except for the distribution to qualified investigators of the antibody to glutamic acid decarboxylase which has been produced in our laboratory and is being used for studies on the distribution of the enzyme in brain.</p>		

Other Professional Personnel Engaged on Project:

Donald E. Schmechel	Medical Officer	LCS NIMH
Wolfgang Oertel	Guest Worker	LCS NIMH
Virginia K. Weise	Chemist	LCS NIMH
Marcel Tappaz	Visiting Fellow	LCS NIMH
Enrico Mugnaini	Chief, Lab of Neuromorphology	State U. Conn.

Methods Employed: The use of the GAD antibody for electron-microscopic visualization of the enzyme was summarized in a review by various coworkers who developed and applied the technique.

Major Findings: No new findings since project was terminated.

Significance to Biomedical Research and to the Program of the Institute: Knowledge of the location and axonal distribution of neurotransmitters is required to understand mechanisms of brain function.

Proposed Course: As indicated in last year's annual report, this project has been terminated.

Publications:

Oertel, W.H., Mugnaini, E., Schmechel, D.E., Tappaz, M.L. and Kopin, I.J.: The immunocytochemical demonstration of gamma-aminobutyric acid-ergic neurons - Methods and application. *Cytochemical Methods in Neuroanatomy* 297-329, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00405-04 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of Physiological Activity of Aminergic Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Medicine		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The objectives of this project are to assess changes in properties (number, affinity) of receptors in response to alterations in the environment, drug administration, stress, or hormones, and to develop methods for assessing receptor function in intact animals, in vivo. This year studies on the role of prostaglandins in regulation of blood pressure implicated in the pathogenesis of the hypertension in experimental animals have continued.</p> <p>Studies of responses in intact, awake animals provide a necessary complement to studies in pithed rats (project Z01 MH 00401).</p> <p>Studies on the modes of action of phencyclidine and lithium which involved dopamine receptor changes have been examined.</p>		
(355)		

Other Professional Personnel Engaged on Project:

Charles R. Saller	Guest Worker	LCS NIMH
Giora Feuerstein	Guest Worker	LCS NIMH
Zofia Zukowska-Grojec	Visiting Fellow	LCS NIMH
Mohamed Bayorh	Visiting Fellow	LCS NIMH
Robert Zerbe	Senior Staff Fellow	LCS NIMH

Objectives: Determining the mechanisms by which receptors are activated and the events which lead to a response. This year the focus has been on the influence of arachidonic acid metabolites - the prostaglandins and leukotrienes on cardiovascular responses and direct examination of dopamine.

Methods Employed: Blood pressure and heart rate responses are studied in intact animals and pithed rats. Dopamine receptors are studied by examining behavioral effects of drugs known to activate dopamine receptors or by binding of dopamine receptor ligands to brain membranes in vitro.

Major Findings: Arachidonic acid is a precursor of prostaglandins (PGs) which are formed as a result of the action of cyclooxygenase and of leukotrienes (LTs) formed by the action of a lipooxygenase. Recently the leukotrienes have been identified as the major components of slow reacting substance of anaphylaxis (SRS-A). Leukotriene D₄ (LTD₄) produces a dual cardiovascular effect in spontaneously hypertensive (SHR) rats which differs from that seen in normotensive rats of the Wistar-Kyoto (WKY) rats from which they were derived. In both SHR and WKY rats LTD₄ evokes a pressor response, but SHR rats are most sensitive to this agent and in SHR but not in WKY rats, the initial pressor response to LTD₄ is followed by a prolonged hypotension and bradycardia which culminates in death. Ca⁺⁺ channel blockade with verapamil diminished the early pressor and late hypotensive effects of LTD₄ and enhanced survival, but β -adrenoceptor blockade, indomethacin treatment (to block PG formation), coenzyme Q (which destroys free radicals and enhances survival in some forms of myocardial ischemia), and FPL 55712, (an SRS-A antagonist) had no significant effect in preventing the LTD₄ deleterious effects on the heart.

LTD₄ prevents the pressor response to sympathetic stimulation in pithed rats, without altering the stimulation-induced plasma NE increase. The LTD₄ also blocks the pressor action of angiotensin and vasopressin, suggesting that it blocks vascular smooth muscle contraction distal to the receptors for these pressor agents.

The pressor response induced by LTD₄ is attended by a marked increase in vascular resistance in the heart, splanchnic area, muscles, and skin without a change in cardiac output. The blood flow to the kidney is not altered - vascular resistance is actually decreased. During the subsequent hypotensive phase, cardiac output is markedly reduced and blood flow to the various organs decreases with severe myocardial ischemia.

Phencyclidine is a widely abused psychoactive drug which appears to involve activation of brain dopaminergic systems. Prolactin secretion is normally inhibited by dopamine; PCP administered for the first time diminishes plasma prolactin, but after 30 days of repeated daily doses of PCP, the drug loses this effect, although motor effects persist (Drs. Lozovsky, Saller, Bayorh and Chiueh).

Chronic PCP treatment lowers slightly, but statistically significantly, ^3H -spiroperidone binding to dopamine receptors.

In following a course of research on changes in dopaminergic neurotransmission with altered glucose levels, it was found that the increase in dopamine receptors in brains of rats with experimental diabetes can be prevented by co-administration of lithium (Drs. Lozovsky, Saller and Kopin). The potential usefulness of this is in treatment of tarditive dyskinesias or the on-off reaction in dopa-treated parkinsonian patients will be investigated in primates.

Significance to Biomedical Research and to the Program of the Institute:

Receptor function alteration is a potential factor in disease states and pharmacological responses. The mode of regulation of receptor function must be further understood to determine how changes in receptors occur in disease or during drug action.

Proposed Course:

Continued investigation of effects of stress, drugs, hormones, etc. on various aspects of receptor function (agonist binding, secondary effects at a biochemical level, e.g., cyclic AMP ion transport and net responses).

Publications:

Feuerstein, G., Zukowska-Grojec, Z., Bayorh, M.A., Krause, M., Utsonomiya, T., Lovenberg, W., and Kopin, I.J.: Effect of arachidonic acid on blood pressure, heart rate and plasma norepinephrine, epinephrine, 6-Keto-PGF₁ and TXB₂ conscious SHR and WKY rats. In Ganten, D. (Ed.): 4th International Symposium on SHR rats. Spontaneous Hypertensive Rats. Stuttgart, W. Germany, Schattauer Verlag, 1982.

Feuerstein, G., Zukowska-Grojec, Z., Krausz, M., Blank, M.L., Snyder, F. and Kopin, I.J.: Cardiovascular and sympathetic effects of 1-O-hexadecyl-2-acetyl-sn-glycero-3-phosphocholine in conscious SHR and WKY rats. Clin. Exp. Hypertens. A4(8): 1335-1350, 1982.

Zerbe, R.L., Bayorh, M.A. and Feuerstein, G.: Vasopressin: An essential pressor factor for blood pressure recovery following hemorrhage. Peptides 3: 509-514, 1982.

Zerbe, R.L., Feuerstein, G., Bayorh, M.A. and Kopin, I.J.: Cardiovascular, sympathetic and renin-angiotensin system responses to hemorrhage in vasopressin deficient rats. Endocrinology 111: 608-614, 1982.

Zukowska-Grojec, Z., Bayorh, M.A., Yaar, I., Feuerstein, G. and Kopin, I.J.: Leukotriene D₄: Divergent cardiovascular and sympathetic effects in SHR and WKY rats. In Samuelsson, B., Paoletti, R. and Romwell, P. (Eds.): Advances in Prostaglandin, Thromboxan and Leukotriene Research. New York, Raven Press, 1983, pp. 407-412.

- Zukowska-Grojec, Z., Bayorh, M.A., Kopin, I.J. and Feuerstein, G.: Leukotriene D₄: Cardiovascular and sympathetic effects in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats. *J. Pharmacol. Exp. Ther.* 223: 183-189, 1982.
- Bayorh, M.A., Zukowska-Grojec, Z., Ezra, D., Feuerstein, G., and Kopin, I.J.: Cardiovascular and sympathetic responses to chronic arachidonate in SHR and WKY rats. *Hypertension* 5: 172-179, 1983.
- Lozovsky, D., Saller, C.F. and Kopin, I.J.: Lithium and the prevention of dopamine receptor supersensitivity in diabetic rats. *Am. J. Psychiatry* 140: 613-614, 1983.
- Lozovsky, D., Saller, C.F., Bayorh, M.A., Chiueh, C.C., Rice, K.C., Burke, T. R. Jr. and Kopin, I.J.: Effects of phencyclidine on rat prolactin, dopamine receptor and locomotor activity. *Life Sci.* 32: 2725-2731, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00274-09 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Methods of Ionization in Mass Spectrometry		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH		
COOPERATING UNITS <i>(if any)</i> Biomedical Engineering and Instrumentation Branch, DRS Department of Pharmacology, George Washington University, Washington, D.C.		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: .5	OTHER: .5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p>An experimental <u>surface ionization tandem mass spectrometer</u> has been constructed and is being tested for the analyses of polar organic compounds in complex mixtures of biological origin. Phospholipids, drug metabolites and small peptides are being partially purified and analyzed by liquid surface ionization techniques.</p> <p>A <u>microwave interface</u> to convert organic compounds eluting from a capillary gas chromatograph to simple di- or triatomic species has been refined and tested on two mass spectrometers with oxygen and hydrogen as reagent gases. Specific nuclides such as ¹³C, ¹⁴C, N, S, halogen, etc. can be detected with high sensitivity.</p> <p>The goal of both mass spectrometric developments is to devise new tools to solve intractable or presently difficult analytical problems in pharmacology and neuroscience.</p>		

PROJECT DESCRIPTION:

Other Professional Personnel Engaged on Project:

Leonid Kelner
Fred P. Abramson

Visiting Scientist
Guest Worker

BEIB, DRS
Professor, Department of
Pharmacology, G.W.Univ.
Wash. D.C.

The collaborative project to design and construct a mass spectrometer suitable for testing ionization methods particularly suited to problems in analytical biochemistry (see Project No. Z01 RS 10073-04 BEI) has resulted in a working instrument. Preliminary tests have defined the energy characteristics of sputtered organic compounds in a glycerol matrix and have demonstrated the utility of an energy analyzer preceding a quadrupole mass filter (Kelner and Markey, cited in project above). This instrument is now being tested for surface analysis of intact phospholipids, drug metabolites, and small peptides from several collaborative research projects. Tandem mass filters are used to select any molecular ion from the sputtered ion mixture, and collisionally induce characteristic fragmentation which can be used for structure analyses.

The microwave discharge interface for detection of specific nuclides in organic compounds eluting from a capillary gas chromatograph which was developed and reported last year, has been further refined. A heatable and tunable cavity was designed and constructed on a low resolution quadrupole instrument, using oxygen as a reactant gas. An alternative approach has been explored employing a high resolution double focusing magnetic mass spectrometer and hydrogen as reactant gas. Approximately equal sensitivities were determined with both systems, but refinements are still being pursued. An ultimate sensitivity of less than 10 dpm ^{14}C per material is expected.

Significance to Biomedical Research: Structure elucidation of unknown compounds in complex mixtures, or the specific detection and quantification of known compounds remain difficult areas of biomedical research. Polar, non-volatile compounds have remained a particular problem. Progress on projects which require these developing analytical methodologies will be substantially speeded, especially in areas of drug metabolism and the determination of unknowns with biological activities.

Proposed Course: Both instrumentation developments will be applied to real problems during the next year (see projects Z01 MH 00279-01 LCS and Z01 MH 00276-04 LCS for example). As required in these experiences, refinements in the analytical equipment will be made.

Publications:

Abramson, F.P. and Markey, S.P.: A chemical reaction capillary gc/ms interface. Proc. 30th Ann. Conf. Mass Spectrometry and Allied Topics, 866-867, 1982.

Markey, S.P. and Abramson, F.P.: Element and isotope specific detection by capillary gas chromatography-mass spectrometry using a microwave discharge interface. In Duncan, W.P. and Susan, A.B. (Eds.): Synthesis and Applications of Isotopically Labeled Compounds. Elsevier, Amsterdam, 1983, pp. 291-296.

Markey, S.P. and Abramson, F.P.: Capillary gas chromatography-mass spectrometry with a microwave discharge interface for radioactive carbon-containing compounds. Anal. Chem., 54, 2375-2376, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00275-06 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Release and Turnover of Catecholamine Metabolites in Human Subjects		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) 		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="height: 400px; border: 1px solid black; margin-top: 10px;"></div>		

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00276-04 LCS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Metabolism of Melatonin

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry

COOPERATING UNITS (if any)

Department of Pediatrics, USUHS

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The major urinary metabolite of the pineal hormone melatonin, 6-hydroxy-melatonin is being quantified by gas chromatography-negative chemical ionization mass spectrometry. Urinary excretion rates of this metabolite are being used to determine the possible role of the pineal gland in human reproductive biology - i.e., its function during pubertal development and throughout the menstrual cycle. A longitudinal study of melatonin metabolite excretion by young girls is in the third year. Each girl has maintained a self-consistent level of excretion, which has not, as yet, correlated with pubertal change. In a study of six menstruating adult women, daily variations in melatonin metabolite excretion were measured for two menstrual cycles. There were no consistent changes with regard to key normal events in each cycle. Day-to-day variations were more significant than those observed in pre-pubertal girls, suggesting either a day-to-day change in pineal function or another unanticipated study variable. A correlative study of plasma radioimmunoassayable melatonin vs. urinary 6-hydroxymelatonin is in progress to resolve this issue. An assay of urinary 5-methoxyindoleacetic acid, a candidate melatonin metabolite, has been developed and applied to human and animal samples. It does not reflect pineal gland activity in man or rat.

Project Description:Other Professional Personnel Engaged on Project:

Sadayoshi Higa	Visiting Fellow	SAB, LCS, NIMH
Merrily A. Poth	Asst. Prof. Pediatrics	USUHS
Miyoshi Fukui	Guest Worker	LCS, NIMH

Objectives: The role of the pineal gland in human physiology remains undefined. Previously, we developed an assay for the major urinary metabolite of melatonin, the conjugated form of 6-hydroxymelatonin. The urinary assay of this metabolite has been shown to be selective and specific for the pineal hormone, varying diurnally in normal primates, and missing from pinealectomized monkeys or neuronally deficient humans. We have applied the urinary assay to determine whether pineal function is related to pubertal development or the menstrual cycle in humans. Both questions require daily monitoring of pineal function which can be achieved best by an integrative measure of the urinary metabolite excretion.

A second area of investigation has been to define alternate pathways of melatonin metabolism. Two postulated urinary metabolites were determined: 5-methoxyindoleacetic acid and N-acetyl-5-methoxykynurenamine. Internal standards and quantitative assays for both compounds were developed and applied.

Methods Employed: Urines were collected from human volunteers in 8-hour aliquots to permit some measure of diurnal variation. Conjugated 6-hydroxymelatonin was quantified using gas-chromatography-negative chemical ionization mass spectrometry. For the assays of other metabolites, deuterated internal standards were synthesized (see Z01 MH 00277-04 LCS) and utilized for gc-ms assays.

Major Findings: The pattern of daily excretion of 6-hydroxymelatonin in young girls studied over a 2-year period have not shown a significant change with pubertal development. Within the next year, each girl will have reached Tanner stage 3. The interim findings are suggestive that the pineal gland produces a constant, but individually distinct, amount of melatonin in pre-menarchal girls. However, in monitoring the melatonin metabolite production from women of child bearing age throughout the menstrual cycle, significant day-to-day differences were observed. Either there is a difference in the two study groups due to menstruation, or there is a problem in the samples collected during the menstrual cycle study. A resolution to this issue is being sought.

In studies in rats, we have demonstrated that urinary 5-methoxyindoleacetic acid (5-MIAA) increases when melatonin is injected; however, the hypothesized kynurenamines could not be detected in rat brain or urine. While melatonin could be metabolized to 5-methoxyindoleacetic acid, it does not appear to be the major source of this minor urinary metabolite. There is no difference in day vs. night or intact vs. pinealectomized rat urinary excretion of this putative metabolite. The levels of 5-MIAA excreted by humans are

characteristic and relatively constant on a day-to-day basis, perhaps reflecting turnover of methylated indoleamines other than melatonin.

Significance to Biomedical Research: Studies on the normal physiologic role of melatonin in human biochemistry are lacking. This project is intended to gather baseline data and define the possible role of melatonin in several of its most frequently cited functions. Postulates regarding altered melatonin production as a consequence of altered circadian function and their relation to mental health require these data.

Proposed Course: Continuation of the longitudinal puberty study and resolution of the menstrual cycle data are major objectives. For the latter, a correlation of radioimmunoassayable plasma melatonin from samples drawn hourly will be made with urinary 6-hydroxymelatonin. If this correlation holds, then the daily metabolite excretion values can be taken to reflect a true day-to-day pineal functional difference.

Publications:

Tetsuo, M., Poth, M., and Markey, S.P.: Melatonin metabolite excretion during childhood and puberty. J. Clin. Endocrinol. Metab. 55: 311-313, 1982.

Markey, S.P. and Buell, P.E.: Pinealectomy abolishes 6-hydroxymelatonin excretion by male rats. Endocrinology, 111: 425-426, 1982.

Taylor, P.L., Garrick, N.A., Burns, R.S., Tamarkin, L., Murphy, D.L., and Markey, S.P.: Diurnal rhythms of serotonin in monkey cerebrospinal fluid. Life Sciences 31: 1993-1999, 1982.

Poth, M., Tetsuo, M., and Markey, S.P.: 6-Hydroxymelatonin excretion during childhood and puberty. In Klein, D.C. (Ed.): Melatonin Rhythm Generating System. Basel, Switzerland, S. Karger, 1982, pp. 204-209.

Poth, M., Higa, S., and Markey, S.P.: The pineal gland and sexual function in man. In Frascini, F. (Ed): Endocrine Function of the Pineal Gland. Plenum Press, in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00277-04 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Synthesis of Stable Isotope Labeled Compounds		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: .4	PROFESSIONAL: .3	OTHER: .1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Stable and some radio isotope labeled compounds have been synthesized to support other laboratory projects. In particular, the possible <u>melatonin metabolites</u> 5-methoxyindole acetic acid (O-CD ₃); N-acetyl-5-methoxy-kynurenamine (O-CD ₃); and their ethyl analogues were prepared. (see Z01 MH 00276-04 LCS). Labeled analogues of <u>N-methyl-4-phenyl- 1,2,3,6 tetra-hydropyridine</u> (N-CD ₃ ; 2,6-D ₂) were prepared as well as 3-H and 14-C analogues (see Z01 MH 00279-01 LCS).		

Project Description:Other Professional Personnel Engaged on Project:

Miyoshi Fukui	Guest Worker	SAB, LCS, NIMH
Benjamin Sklarz	Guest Worker	SAB, LCS, NIMH

Objectives: The synthesis of labeled compounds is an integral support function to investigations of metabolism and distribution of endogenous and xenobiotic compounds. During the past year, efforts have been focused on melatonin metabolites (Z01 MH 00276-04 LCS) and labeled variants of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP, Z01 MH 00279-01 LCS).

Methods Employed: Conventional methods of chemical syntheses employing isotopes have been utilized.

Major Findings: The melatonin metabolites 5-methoxyindoleacetic acid (O-CD₃) N-acetyl-5-methoxykynurenamine (O-CD₃) as well as their ethyl analogues were prepared for use in melatonin metabolism studies. Labeled analogues of NMPTP (N-CD₃; 2,6, D₂; N-CD₃ and 2,6 D₂) were prepared. In order to perform binding and metabolism studies, tritiated NMPTP (2,6 ³H₂) and carbon labeled NMPTP (1-¹⁴C) were also prepared. General methods of synthesis have produced methods which are suitable for analytical determinations of NMPTP and some of its oxidized metabolites.

Significance to Biomedical Research: The availability of labeled compounds is frequently the limiting step in metabolism projects. A program in analytical biochemistry requires continuing synthetic efforts to prepare stable and radioisotope analogues for the timely and efficient solution to metabolism projects.

Proposed Course: Most synthetic efforts will be directed toward yet-to-be elucidated NMPTP metabolites. Sufficient quantities to permit toxicity testing in rodents and primates will be prepared.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00279-01 LCS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacology of Neurotoxins

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Sanford P. Markey, Chief, Section on Analytical Biochemistry, LCS, NIMH

COOPERATING UNITS (if any)

Section on Experimental Therapeutics, LCS, NIMH

Section of Histopharmacology, LCS, NIMH

Office of the Chief, LCS, NIMH

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Analytical Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, MD 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
- ☐ (a1) Minors
- ☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The mechanism of action of the neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (NMPTP) is being studied in several animal species. In primates, NMPTP produces a persistent parkinsonian syndrome. Neurochemical studies have demonstrated a close correspondence between idiopathic Parkinson's disease in humans and NMPTP toxicity. Depletion of dopamine from the putamen and caudate nucleus is most pronounced, dropping to 3-5% of pre-NMPTP treatment levels. However, in the rat, guinea pig, and cat, equivalent chronic neurotoxicity cannot be produced. Acute pharmacological effects of high doses of NMPTP have been observed in these species, but even chronic NMPTP treatment diminishes dopamine content by only 40-60% in the nigro-striatal system of the guinea pig. Radiolabelled NMPTP has been prepared to determine the pattern its metabolism in several animal species. Autoradiographic as well as tissue punch techniques have demonstrated the selective localization of NMPTP in the caudate nucleus and putamen of the monkey brain, but its non-selective distribution in rat and guinea pig brain. The high pressure liquid chromatographic pattern of brain metabolites formed from labelled NMPTP is species specific.

The determination of the toxic metabolic path in the monkey is being studied to learn how a peripherally administered, relatively small organic molecule can produce a specific neurochemical lesion, and whether this mechanism is relevant to idiopathic Parkinson's disease.

Project Description:Other Professional Personnel Engaged on Project:

Chuang Chieuh	Senior Staff Fellow	SAB, LCS, NIMH
Jan Johannessen	Staff Fellow	SAB, LCS, NIMH
Richard S. Burns	Senior Staff Neurologist	SET, LCS, NIMH
Irwin J. Kopin	Chief	LCS, NIMH
David Jacobowitz	Chief	SH, LCS, NIMH
Miles A. Herkenham	Research Psychologist	LN, NIMH

Objectives: The neurotoxicity of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (NMPTP) in man and monkey has been described in the past year. Relatively small amounts (.3-.5 mg/kg) administered peripherally over five days produce a parkinsonian syndrome in primates which neurochemically and histopathologically resembles idiopathic Parkinson's disease in humans. An animal model of this disorder has long been sought, but other lesioning techniques have failed to produce a persistent condition with all of the neurochemical and locomotor effects observed in the human disorder and NMPTP exposure. The mechanism of this neurotoxicity is particularly interesting in that larger doses of NMPTP given chronically to rats, cats; or guinea pigs fail to produce any observable locomotor deficit, although measurable diminution of dopamine in the nigro-striatal system is apparent in the rat (40% decrease after 28 doses of 5 mg/kg over 28 days) and the guinea pig (50% decrease after 21 doses of 10 mg/kg over 21 days). The objectives of this project are to unravel the mechanism of NMPTP neurotoxicity in the primate, particularly with regard to its specific effects on the caudate nucleus and putamen, and lack of effect on the nucleus accumbens.

Methods Employed: NMPTP toxicity is being studied by qualitative and quantitative observation of animal behavior and locomotion; neurochemical determination of catecholamines and their metabolites in specific brain regions by high pressure liquid chromatography (hplc) with electrochemical detection; determination of the pattern of NMPTP distribution, metabolism, and excretion, using radio and stable isotope labelled NMPTP (^3H , ^{14}C , ^2H) in several animal species; identification of metabolites unique to the primate and synthesis and pharmacological testing of candidate metabolites. These methods rely upon high specific activity NMPTP prepared in this study to characterize by hplc labeled metabolites extracted from physiological fluids or tissues. Autoradiography is being employed to study tissue localization. The structures of isolated metabolites are being determined by mass spectrometry, and will be synthesized and tested *in vivo* to measure their neurotoxicity. Receptor binding studies of NMPTP and NMPTP metabolites with respect to known or other specific receptors is being pursued.

Major Findings: NMPTP produces markedly different effects in primates and rodents. Whereas a persistent parkinsonism results from 5 daily injections of 0.3 mg/kg in monkeys, no obvious persistent locomotor effects were observed in rats or guinea pigs injected daily for 28 or 21 days, respectively. Neurochemical analysis revealed dopamine content diminished by 95% in the striatal system of the monkey, but only 40-50% in the chronically treated rat and guinea pig. A single monkey was injected with tritiated NMPTP, and the pattern of its distribution and excretion over 24 hours compared to that of rodents injected and sacrificed at various times. Rodents metabolize and excrete NMPTP more rapidly, although detectable radioactivity persisted in the guinea pig for more than nine days. Chromatographic patterns of soluble metabolites from rodent brain were considerably different from those of monkey brain. A single major metabolite is formed and stored in monkey brain. The greatest concentration of this metabolite is found in the striatal pathway, suggesting it is responsible for the observed neurotoxicity.

Significance to Biomedical Research: The NMPTP lesioned primate is the best animal model of Parkinson's disease which has been described. The mechanism of action of NMPTP may be relevant to the cause of idiopathic Parkinson's disease. The blockade of NMPTP action may suggest methods to slow the disease progress. A primate specific metabolite may prove neurotoxic in the rodent and provide a useful animal model for therapeutic testing of a range of pharmacological agents.

Proposed Course: Characterization and synthesis of the primate specific NMPTP metabolite will be used to test its neurotoxicity in rodents and the primate. The mechanism of axonal degeneration will be studied using NMPTP and its major metabolite *in vitro*. Blockade of NMPTP toxicity will be tested in the primate. Quantitative assay of the NMPTP metabolite will be used to determine its lifetime in primate brain. Antibodies to the NMPTP metabolite will be prepared and used to determine whether a similar species is present in brains of patients who have died of idiopathic Parkinson's disease. Studies of the neuropharmacology of lesioned animals treated with L-Dopa will be pursued to test observations from clinical experience such as dopamine supersensitivity, the electrophysiology of movement initiation; and "on-off" and "freezing" episodes.

Publications:

Chiueh, C.C., Burns, R.S., Markey, S.P., Jacobowitz, D., Ebert, M.H., and Kopin, I.J. Effects of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a cause of an extrapyramidal syndrome in man, on the nigrostriatal dopaminergic system in the rat, guinea pig and monkey. Abs. Catecholamine Symposium Goteborg, Sweden, June 1983, in press.

Chiueh, C.C., Markey, S.P., Burns, R.S., Johannessen, J., Jacobowitz, D.M., and Kopin, I.J. N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a parkinsonian syndrome causing agent in man and monkey, produces different effects in guinea pig and rat. Abs. Amer. Soc. Pharm. Exp. Therapeutics, Phila., Pa., 1983, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00280-01 LCS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies in Morphine Tolerance		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jan Johannessen, Staff Fellow, LCS, NIMH		
COOPERATING UNITS (if any) Department of Physiology, Medical College of Virginia, Richmond, VA		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Analytical Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: .5	PROFESSIONAL: .5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Infusion of cerebrospinal fluid from morphine tolerant rats into naive rats renders the recipients less susceptible to the acute analgesic effects of morphine as measured by tail-flick latency response. The metabolites of morphine-tolerant vs. acutely treated rats have been studied to determine if this phenomenon is related to a brain specific metabolite. No differences were detected in <u>cerebrospinal fluid opiate receptor</u> binding capacity between the animal groups. Tolerant animals had significantly less conjugated morphine in both csf and urine than acutely treated animals. </p> <p> The principle in the csf of morphine-tolerant rats responsible for blunting the morphine analgesic response in naive animals is likely to be an endogenous agent. </p>		

Project Description:Other Professional Personnel Engaged on Project:

Sanford P. Markey
D.J. Mayer

Chief
Prof. of Physiology

SAB, LCS, NIMH
Med. College of VA.

Objectives: Infusions of cerebrospinal fluid from morphine tolerant rats into naïve rats renders the recipients less susceptible to the acute analgesic effects of morphine. One possible explanation is the generation of a morphine metabolite with antagonistic activity. We therefore studied the metabolites of morphine tolerant and acutely treated rats to determine if there was a difference in metabolites which could account for the phenomenon.

Methods Employed: Metabolism of morphine in tolerant and non-tolerant rats was studied using in vivo injections of morphine ring-labelled with tritium. Cerebrospinal fluid and other body fluids were drawn from anesthetized animals and frozen. The distribution of morphine metabolites was assessed using thin layer chromatography followed by scanning for tritium radioactivity. Absolute levels of free morphine were measured on GC-MS. Opiate binding activity was estimated by a radioreceptor assay, utilizing tritiated naloxone as a ligand.

Major Findings: We did find significant differences in the pattern of metabolites between tolerant and acutely treated rats. Within the CSF, the levels of free morphine in tolerant and acutely treated rats as measured by GC-MS were similar, but the relative proportion of conjugated forms was greatly reduced in the tolerant animals. The urine of tolerant animals showed a similar enrichment in free morphine as compared to the conjugated forms. Despite these changes in morphine metabolism observed in the tolerant state, opiate binding assays revealed no difference in the opiate receptor binding capacity of CSF taken from tolerant or acutely treated animals.

Significance to Biomedical Research: The significance is twofold: 1) it eliminates a morphine metabolite as the agent responsible for the transference of morphine tolerance, thus indicating an endogenous agent was responsible; and 2) our finding of a change in the metabolism of morphine in the tolerant state is contrary to the classical literature.

Proposed Course: The initial objective has been achieved and therefore no more studies aimed at looking for antagonistic morphine metabolites are contemplated. We do intend to explore further the differences in morphine metabolism seen between tolerant and acutely treated rats. By switching to more sensitive HPLC separations and utilizing GC-MS assays for morphine metabolites, we will be able to express these differences quantitatively.

Publications:

Lu, G.Q., Johannessen, J.N., and Mayer, D.J.: Morphine tolerance induces an endogenous opiate antagonist in cerebrospinal fluid. Soc. Neurosci. Abstr., 8 (1982) p. 778.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00351-09-LCS

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Pharmacology of the Central Nervous System

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation) Michael H. Ebert, Chief, Section on Experimental Therapeutics, LCS, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this study is to develop techniques for studying central nervous system monoamine metabolism in man, and to carry out pharmacokinetic studies of psychoactive drugs. Studies of the kinetics and clearance of deuterated homovanillic acid (HVA) from the circulation continued in Rhesus monkeys and patients. Problems with the application of this technique to study CNS dopamine turnover in man were resolved by further study of the kinetics and volume of distribution of the metabolite, HVA in plasma and cerebrospinal fluid.

R.S. Burns

Staff Neurologist

LCS NIMH

Project Description:

Objectives: (1) To develop techniques for studying parameters of central nervous system monoamine metabolism in man. (2) To carry out pharmacokinetic studies of psychoactive drugs. (3) To utilize these techniques to study patients with a variety of psychiatric, neuropsychiatric, and psychosomatic disorders.

Methods Employed: (1) Biochemical methods: fluorometric, gas chromatographic, and mass spectrometric methods are used for assay of endogenous catecholamine metabolites in tissues and body fluids. Mass spectrometric methods are used for assay of deuterium-labelled catecholamine metabolites administered exogenously or synthesized endogenously. Gas chromatographic and radio-immunoassay techniques are used for assay of drugs in body fluids. (2) Physiological methods: As the project progresses, an increasing number of metabolic experiments will be carried out in patients. However, as new procedures are being developed, appropriate experiments will be carried out in rat brain tissue and in primates using serial sampling of blood, cerebrospinal fluid, and urine as necessary. To facilitate these preclinical in vivo experiments on brain metabolism, a small primate metabolic laboratory has been developed with a capacity to maintain chronic collection of lateral ventricle or lumbar cerebrospinal fluid from chaired Rhesus monkeys for days and weeks at a time.

Major Findings: Unfortunately, the laboratory work in the section was comprised this year by the ADAMHA hiring freeze and RIF which eventually eliminated the laboratory support personnel in the section. The work on this project was diminished as a result and will be resumed next year.

The occurrence of free and conjugated dopamine was determined by high performance liquid chromatography with electrochemical detection in brain areas, peripheral tissues, and CSF from Rhesus monkeys. Free norepinephrine, epinephrine, and 3,4-dihydroxyphenylacetic acid were also determined in some tissues. Conjugated dopamine was found to be widely, but not homogeneously, distributed in this species. In the brain, conjugated dopamine was found to account for greater than ten percent of the total dopamine present in the frontal cortex (74%), cingulate gyrus (31%), cerebellum (16%), and occipital cortex (11%). Conjugated dopamine accounted for 21% of the total dopamine present in the frontal cortex (11%). Conjugated dopamine accounted for 21% of the total dopamine in the liver, and ranged from 10 to 20% of the total in testicle, kidney, and heart. In CSF from both the lateral ventricle and lumbar thecal sac, free dopamine was not reliably detected, but conjugated dopamine was found in all samples tested.

Significance of Biomedical Research: Assessing the rate of amine metabolite formation in animals and in patients provides information on the rate of amine utilization. The development of more quantitative methods of determining central nervous system amine utilization in man is essential for testing hypotheses regarding the role of amines psychiatric, medical, and neurological disorders, and assessing the effects of drugs on amines in the brain.

Z01-MH-00351-09-LCS

Proposed Course: The metabolic techniques described above for the study of catecholamine metabolism are now being applied to studies in normal volunteers and patient groups.

Publications:

Elchisak MA, Powers KH, Ebert MH: Demonstration of Conjugated Dopamine in Monkey CSF by Gas Chromatography-Mass Spectrometry. J Neurochem 39(3):726-728, 1982.

Elchisak MA, Cosgrove SE, Ebert MH, Burns RS: Distribution of Free and Conjugated Dopamine in Monkey Brain, Peripheral Tissues, and Cerebrospinal Fluid Determined by High Performance Liquid Chromatography. Brain Res, In press.

Ebert MH, Kopin IJ, Gordon EK, Markey SP, and Blombery P: 3-Methoxy-4-Hydroxyphenylglycol in Plasma Defects Overall Norepinephrine Metabolism in Humans. J Neurochem, In press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01-MH-00352-08 LCS

PERIOD COVERED

October 1, 1982 - September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological and Psychometric Studies of Neuropsychiatric Syndromes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Michael H. Ebert, Chief, Section on Experimental Therapeutics, LCS NIMH

COOPERATING UNITS (if any)

Section on Medicine, LCS; Laboratory of Psychology and Psychopathology, NIMH; Laboratory of General and Comparative Biochemistry, NIMH; NIAAA

LAB/BRANCH

Laboratory of Clinical Science

SECTION

Section on Experimental Therapeutics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Several neuropsychiatric syndromes including Alzheimer's dementia and Korsakoff's psychosis are being studied in terms of memory function and pharmacological treatment. The pathophysiology and neuropharmacology of anorexia nervosa, bulimia, and essential tremor are also under investigation. Underweight anorectics have a subnormal and erratic release of vasopressin (AVP) to intravenous hypertonic saline. These abnormalities were associated with an increase in the level of AVP in CSF. Patients with anorexia nervosa who successfully maintain weight after refeeding may do so by changes in energy balance mediated by central and peripheral adrenergic systems. Clonidine hydrochloride was not found efficacious in the treatment of the memory disorder of Korsakoff's psychosis. No evidence was found for alterations in central nervous system metabolism of norepinephrine in Korsakoff's psychosis.

W.H. Kaye	Staff Psychiatrist	LCS NIMH
P.R. Martin	Visiting Scientist	NIAAA
D. Goldman	Clinical Associate	LCS NIMH
C.R. Merrill	Sr. Research Scientist	LGCB NIMH
H. Weingartner	Research Psychologist	LPP NIMH
I.J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
L.E. Nee	Clinical Social Worker	LCS NIMH
R.S. Burns	Staff Physician	LCS NIMH
S.J. Weiss	Psychologist	LCS NIMH
H.E. Gwirtsman	Medical Staff Fellow	LCS NIMH

Project Description:

Objectives: The purpose of this study is to investigate several neuropsychiatric syndromes from the perspective of memory and intellectual function, neurotransmitter metabolism, neuroendocrine function, and possible pharmacological treatment. Currently under study are several dementias with known neuropathology, particularly Alzheimer's dementia and Korsakoff's dementia. Other diseases under intensive study are anorexia nervosa, Parkinson's disease, and essential tremor.

Methods: Methods for studying information processing in neuropsychiatric disorders include learning tasks that permit the assessment of stimulus and modality specific information in processing (1) short-term memory; (2) long-term memory; (3) retrieval processing; (4) encoding; (5) the interrelationships of these processes; and (6) factors that might influence decay and interference of memory in these systems. Procedures are used to test free recall, serial learning, cued recall, and recognition memory.

One strategy for identifying neurotransmitter systems that may play a role in the pathogenesis of psychiatric or neurological symptoms is to study the clinical effects of a series of drugs having relatively specific stimulating or blocking effects on a particular neurotransmitter system. This approach has been profitably applied to research on affective disorders. We are pursuing a pilot investigation of this nature in patients with dementias of varying etiologies, focusing on the adrenergic and cholinergic system.

Methods used in neuroendocrine protocols are established techniques of studying plasma prolactin and growth hormone responses to the acute administration of drugs known to affect dopaminergic neurotransmission. From these endocrine responses, one can infer the functional state of dopaminergic transmission in the tuberoinfundibular systems. Methods used in Stage II drug trials are established methods for the evaluation of psychotropic drugs including double-blind design, appropriate rating scales, and placebo controls.

Methods used in studies of neurotransmitter metabolism are those methods outlined in project Z01 MH 00351-08 LCS.

Methods used to study protein abnormalities or search for genetic markers in psychiatric and neurological diseases involved the application of two-dimensional polyacrylamide gel electrophoresis of proteins. These two-dimensional gels can be studied and quantitatively analyzed either by silver staining of the proteins present or by labeling the proteins in biological

sample and performing radioautograms. Image processing computer systems are used to identify and quantitate the individual proteins appearing on the gel.

Major Findings: Clinical studies of anorexia nervosa have continued to focus on neurochemical disturbances that occur during the evolution of this disease. A clinical design is utilized in which we study anorectics while underweight, throughout weight recovery, and at intervals after weight restoration. In collaboration with Drs. Gold and Robertson (U. of Chicago), we have found that underweight anorectics have a subnormal and erratic release of vasopressin (AVP) to intravenous hypertonic saline. This abnormality persisted in patients studied 3 to 4 weeks after weight recovery, but disappeared in 5 of 7 patients studied after at least 6 months of weight recovery. Abnormalities in the osmoregulation of plasma AVP were almost always associated with an absolute increase in the level of AVP in the cerebrospinal fluid (CSF) or a reversal of the normal CSF/plasma AVP ratio. These results indicate that most if not all patients with anorexia nervosa have abnormal levels of AVP in the plasma and CSF that are corrected very slowly with weight gain. Another intriguing finding is that anorectics, who are food restricters, appear to have higher concentrations of CSF 5-HIAA after probenecid than do anorectics that binge and vomit or use laxatives.

Further studies are underway in investigating norepinephrine (NE) metabolism and its effects on energy utilization in anorexia nervosa. In collaboration with Drs. Jimerson and Lake, we have found no change in mean levels of CSF NE, MHPG, DHPG, or plasma MHPG between underweight anorectics and the same women within 4 weeks after weight recovery. Underweight anorectics eat a mean of 33 cal/kg/day when maintaining a stable weight. In the month after weight is recovered, anorectics eat a mean of 52 cal/kg/day, maintaining a stable weight. Long term weight recovered subjects had significant decreases in CSF NE, MHPG, DHPG, and plasma NE and MHPG compared to controls and a decrease in caloric intake to a mean of 27 cal/kg/day. Thus subjects able to maintain weight long term had a normalized caloric intake associated with a decrease in NE metabolism. Patients with anorexia nervosa who successfully maintain weight after refeeding may do so by changes in energy balance mediated by central and peripheral adrenergic systems.

Studies in this past year have focused on understanding why it is so difficult for anorectics to gain weight and maintain weight recovery. Preliminary data suggest that when refeed, anorectics convert caloric intake to heat production rather than into fat stores or into manufacturing other tissues. This has been documented by 24 hour core temperature recordings, infrared thermography, and BMR measurements. This pattern of excess heat production during refeeding is in contrast to animal studies that tend to find that animals refeed after weight loss have increased metabolic efficiency. Adrenergic pathways are prime candidates as a mechanism of these changes in heat production because of their role in regulating thermogenesis, nutrient utilization, and appetitive behavior. We have nearly completed a study where we have measured plasma NE and NE metabolites and urinary NE metabolites at 2 week intervals during weight recovery as well as in separate groups of anorectics at stages after weight restoration. We have also obtained blood to measure other substances that affect thermogenesis such as endorphins and hormones in the thyroid axis.

A psychological study of normal weight bulimics was completed this year. Psychological and behavioral characteristics were compared in 15 bulimic women and 15 controls matched for age, IQ, and socioeconomic status. Although

physical traits, life stress, family demographics, and incidence of family pathology were similar in both groups, bulimics demonstrated significantly higher levels of psychological pathology and impulsive behavior such as suicide attempts, stealing episodes, drug use, depression, anxiety, obsessive-compulsive traits, low self-esteem, and feelings of helplessness.

The effect of oral clonidine (600 mg per day) on tremor amplitude measured with an accelerometer was studied in 6 patients with essential tremor. Three patients showed a marked decrease (60 - 70%) and 2 patients a mild reduction (9 - 11%) in tremor amplitude accompanied by mild asymptomatic reductions in arterial blood pressure and heart rate, and mild transient side effects. A decrease in the availability of norepinephrine at α -receptors in the brain may account for the effect of clonidine on tremor amplitude. Clonidine, an α -agonist with a different mechanism of action than the widely-used β blockers, represents a therapeutic alternative that may prove to be important in the treatment of patients unresponsive to propranolol and in patients with asthma or diabetes mellitus.

In an effort to define the relationship between idiopathic Parkinson's disease and dementia, cognitive testing was performed in 6 Parkinsonian patients who had not previously received L-dopa or ergot compounds. Unlike patients with progressive dementias such as Alzheimer's disease, untreated Parkinsonian patients with mild-to-moderate motor impairment (Haehn and Yahr State I - IV) are efficient at accessing previously acquired knowledge. They also can learn and remember information that can be processed "automatically" using operations requiring little cognitive capacity. However, these patients demonstrate distinctive impairments requiring effort-demanding cognitive processes.

We have attempted to replicate previous reports of impaired central noradrenergic activity and memory enhancement with the α_2 -noradrenergic agonist clonidine hydrochloride in patients with Korsakoff's psychosis (KP). We could not demonstrate a difference in lumbar cerebrospinal fluid (CSF) concentrations of norepinephrine, its major metabolite, 3-methoxy-4-hydroxyphenyl glycol (MHPG), nor the contribution of plasma to CSF free MHPG in 6 patients with clinically well characterized KP and 8 age-matched healthy normal volunteers. Likewise, CSF concentrations of the major metabolites of dopamine (homovanillic acid) and serotonin (5-hydroxyindoleacetic acid) were no different in patients than in controls. In 7 patients with KP, clonidine treatment did not produce significant changes in measures of either semantic or episodic memory.

During the above study, seven normotensive patients with KP were treated with 2 μ g/kg/day clonidine (C) three times daily for 1 week. Four patients received 12 μ g/kg/day during the subsequent week; three developed hypotensive symptoms at this dose and remained on 6 μ g/kg/day. During a predrug placebo period and after 60 hr. on each dose of C, urinary excretion rates of catecholamine metabolites were determined. C, 6 μ g/kg/day, reduced the urinary excretion of NE metabolites; normetanephrine (NM), vanillylmandelic acid (VMA), and MHPG. The excretion of normetanephrine (NM) was not reduced significantly. The ratio M/NM and M(VMA + MHPG) increased, indicating C's effects are primarily noradrenergic. Reduction in NM/(VMA + MHPG) indicates disproportionate lowering of the O-methylated metabolite of NE compared to its deaminated metabolites, consistent with C inhibition of NE release.

A clinical genetic study of a Canadian family containing 51 members affected with Alzheimer's disease was published this year. This is considered to be the largest pedigree of familial Alzheimer's disease published to date, and includes 4 cases documented by neuropathological findings at autopsy. Two

dimensional electrophoresis of proteins of lymphoblasts, fibroblasts, erythrocytes, plasma, and cerebrospinal fluid is being carried out in family members to search for genetic linkage with previously identified protein polymorphisms visualized on the gels. A search is also being conducted for abnormal proteins related to the disease state. To date fibroblast cell lines have been established from 70 individuals in the family. Utilizing two dimensional electrophoresis and computer assisted visual image analysis, polymorphisms have been identified of 6 plasma proteins, 4 erythrocyte proteins, and 16 fibroblast proteins. Mathematical analysis for linkage of these polymorphisms to Alzheimer's disease is underway.

Significance to Biomedical Research: In recent years interest has increased in the biochemical aspects of learning and memory. Neuropharmacological aspects of memory function have also become a focus of interest. Most of the published studies in this area are in animals. We hope to extend knowledge about pharmacological and biochemical aspects of memory function by studying several diseases in which disordered memory is a primary symptom, and in which there is existing knowledge about anatomical and biochemical pathology. Anorexia nervosa is another neuropsychiatric syndrome in which there is little published information concerning pharmacological treatment and neurotransmitter metabolism. It is clearly established in the animal literature that monoamine systems in the hypothalamus play an important role in the normal regulation of appetite.

Proposed Course: Clinical applications of two-dimensional electrophoresis will include further family studies to describe additional polymorphisms that can be used for linkage analysis. Two-dimensional electrophoresis of cerebrospinal fluid will be applied to the study of several genetic or degenerative neurological diseases. Neurochemical studies of opiate, noradrenergic, and central vasopressin systems in anorexia nervosa will continue with the goal of determining whether these systems play a role in the chronic course of the illness. Studies of bulimia, a parallel disorder of eating behavior, have begun. Pilot neurochemical studies of Parkinson's disease and essential tremor described above will be concluded.

Publications:

Weiss SR, Ebert MH: Psychological and Behavioral Characteristics of Normal Weight Bulimics and Normal Weight Controls. *Psychosom Med*, 45(4):293-303, 1983.

Nee LE, Polinsky RJ, Ebert MH: Tourette Syndrome: Clinical and Family Studies. In, Gilles de la Tourette Syndrome, Friedhoff AJ and Chase TN (Eds), Raven Press, New York, 291-296, 1982.

Caine ED, Polinsky RJ, Ludlow CL, Ebert MH, Nee LE: Heterogeneity and Variability in Tourette Syndrome. In, Gilles de la Tourette Syndrome, Friedhoff AJ and Chase TN (Eds), Raven Press, New York, 437-442, 1982.

Ludlow CL, Polinsky RJ, Caine ED, Bassich CJ, Ebert MH: Language and Speech Abnormalities in Tourette Syndrome. In, Gilles de la Tourette Syndrome, Friedhoff AF and Chase TN (Eds), Raven Press, New York, 351-362, 1982.

Martin PR, Ebert MH, Gordon EK, Kopin IJ: Urinary Catecholamine Metabolites and Effects of Clonidine in Patients with Alcohol Amnestic Disorder. *Clin Pharmacol Ther* 33(1):19-27, 1983.

Small A, Teagno L, Madero J, Gross H, Ebert M: A Comparison of Anorexics and Schizophrenics on Psychodiagnostic Measures. *Journal of Eating Disorders*. 1(3): 49-56, 1982.

Nee LE, Polinsky RJ, Eldridge R, Weingartner H, Smallberg S, Ebert M: A Family with Histologically Confirmed Alzheimer's Disease. *Arch Neurol*. 40(4):203-208, 1983.

Kraemer GW, Ebert MH, Lake CR, McKinney WT: Neurobiological Measures in Rhesus Monkeys: Correlates of the Behavioral Response to Social Separation and Alcohol. In, Symposium on Stress and Alcohol, Brick J and Pohorecky LA (Eds), Elsevier, NY, 171-184, 1983.

Lowenstein RJ, Weingartner J, Gillin JC, Kaye W, Ebert M, Mendelson, W: Disturbances of Sleep and Cognitive Functioning in Patients with Dementia. *Neurobiol Aging*. 3:371-377, 1982.

Gross H, Ebert MH, Faden VB, Goldberg SC, Kaye WH, Caine ED, Hawks R, Zinberg N: A Double Blind Trial of Delta-9-Tetrahydrocannabinol in Primary Anorexia Nervosa. *J Clin Psychopharmacol*. 3(3):165-171, 1983.

Burns RS, Larsen TA, Martin PR, Ebert MH: Clonidine in Essential Tremor, *Lancet*, In press.

Kaye WH, Ebert MH, Lake CR, Raleigh M: Cerebral Monoamine Metabolism in Anorexia Nervosa, *Arch Gen Psychiat*, In press.

Martin PR, Ebert MH, Gordon EK, Weingartner H, Kopin IF: Clonidine Withdrawal in Patients with Alcohol Amnestic Disorder: Relation of Physiologic and Behavioral Changes to Catecholamine Metabolism. *Arch Gen Psychiat*, In press.

Martin PR, Weingartner H, Gordon EK, Burns RS, Linnoila M, Kopin IJ, Ebert MH: CNS Catecholamine Metabolism and Negative Therapeutic Trial of Clonidine in Korsakoff's Psychosis, *Annals of Neurology*, In press.

Kraemer GW, Ebert MH, Lake CR, McKinney WR: Early Social Deprivation: A Possible Etiology for Long-Lasting Hypersensitivity to d-Amphetamine. *Psychopharmacology*, In press.

Ebert MH, Goldman D, Nee LE: Clinical Research in Neuropsychiatry. *Hosp Community Psychiatry*, In press.

Caine ED, Ludlow CL, Polinsky RJ, Ebert MH: Provocative Drug Testing in Tourette Syndrome. *J Amer Acad Child Psychiatry*, In press.

Kaye WH, Lake CR, Gross H, Ebert MH: Catecholamine Metabolism in Anorexia Nervosa. In, Norepinephrine: Clinical Aspects. Lake CR and Ziegler MG (Eds), Williams and Wilkins, Baltimore, MD, In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01-MH-00353-01-LCS
PERIOD COVERED October 1, 1983 - September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical and Pharmacological Studies of Parkinsonism and Related Diseases		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) R.S. Burns, Senior Staff Neurologist, Section on Experimental Therapeutics, LCS, NIMH		
COOPERATING UNITS (if any) Section on Lab. Animal Medicine and Care, IRP, Section on Analytical Biochemistry, LCS; Sec. on Histopharmacology, LCS; Lab. of Cerebral Metabolism, IRP.		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Experimental Therapeutics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.8	PROFESSIONAL: 1.6	OTHER: 1.2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The kinetics of deuterated-homovanillic acid (HVA) were studied in <u>untreated parkinsonian patients</u> to provide an estimate of the central (brain) contribution to the total body production rate of HVA. The relative value of the level of HVA in CSF, blood and urine as an index of brain dopamine metabolism was assessed. <u>Biochemical studies of central and peripheral monoamine systems</u> were carried out <u>in patients with parkinsonism induced by NMPTP (N-methyl-4-phenyl-1,2,3,6-tetrahydrophridine)</u>. The clinical response of these patients to L-dopa and direct acting dopamine agonists was also studied. <u>A new primate model of Parkinson's disease, the NMPTP-lesioned monkey, was developed.</u> The neurochemical and neuropathological changes as well as the abnormal motor behavior produced by NMPTP in the monkey were described.</p>		

Other Professional Personnel Engaged on Project:

M.H. Ebert	Chief, Sec. on Experimental Therapeutics	LCS NIMH
I.J. Kopin	Chief, Lab. of Clinical Science	LCS NIMH
J.M. Phillips	Chief, Sec. on Lab. Animal Medicine and Care	IRP NIMH
S.P. Markey	Chief, Sec. on Analytical Biochemistry	LCS NIMH
D.M. Jacobowitz	Chief, Sec. on Histopharmacology	LCS NIMH
L. Sokoloff	Chief, Lab. of Cerebral Metabolism, DBBR	IRP NIMH

Project Description:

Objectives: (1) To develop techniques for measuring brain dopamine metabolism (turnover) in man. (2) The development of an animal model of Parkinson's disease.

Methods: (1) The plasma clearance rate, pattern of metabolism, and urinary excretion kinetics of deuterated-HVA were studied in man using a gas chromatography-mass spectrometry assay method. (2) The total body production rate of HVA was estimated by measurement of the 24 hr urinary excretion rate of HVA and also derived from the plasma clearance rate of deuterated-HVA and the steady state plasma levels of endogenous HVA. Untreated parkinsonian patients were compared to normals to estimate the fraction originating from the brain. (3) The relative value of the 24 hr urinary excretion rate of HVA and its level in plasma and CSF as an index of brain dopamine metabolism was assessed by comparing untreated parkinsonian patients with normals. (4) Young drug abusers who developed parkinsonism after the intravenous use of an illicit synthetic analgesic preparation were examined clinically and the levels of biogenic amine metabolites in CSF, urine, and plasma were measured to assess the similarity of this syndrome and idiopathic Parkinson's disease. Samples of the illicit drug preparation were analyzed by gas chromatography-mass spectrometry to identify the chemical constituents. (5) NMPTP was administered intravenously to Rhesus monkeys with various dosage schedules. Neurological effects were observed and documented on videotape. Acute changes in the concentrations of biogenic amine metabolites in ventricular CSF were monitored using animals with chronic indwelling lateral ventricular cannulae in place. NMPTP-treated animals were sacrificed and the acute changes in regional levels of biogenic amines and their metabolites and the pattern of catecholamine histofluorescence examined. Neuropathological examination of stained sections of brains from NMPTP-treated animals was also performed.

Major Findings: About 20% of HVA present in the urine is in the form of a conjugate hydrolyzable by gluculase. The individual variation in the percent of conjugated HVA in urine is large (3-42%) which necessitates measurement of total HVA (free + conjugated). The 24 hr urinary excretion rate of HVA (238 μ g/hr) in normals appears to be an accurate measure of the total body production rate of HVA as determined by the plasma kinetics of deuterated-HVA (227 μ g/hr). Although the patients exhibited extrapyramidal signs sufficient to make the diagnosis of Parkinson's disease and the mean CSF HVA level in the patient group (36.5 ± 4.5 ng/ml) was lower than in normals (46.3 ± 2.5 ng/ml) the HVA production rates (derived from plasma kinetics of deuterated-HVA in the 3 patients and 3 normals analyzed to date were not different. This may reflect the fact that these patients, selected because they had not previously received any drug treatment, had early, mild (stage I-II) parkinsonism.

NMPTP-induced parkinsonism in man and idiopathic Parkinson's disease exhibit the same clinical signs (akinesia, rigidity, resting tremor, flexed posture) and these signs are reversed by L-dopa. We have shown that a similar neurological disorder can be produced in the monkey. Akinesia, rigidity, a flexed posture, and a postural tremor which can be reversed by the administration of L-dopa are evident after several intravenous doses of NMPTP. NMPTP acutely affects brain dopamine systems and produces a decrease in the level of HVA in the brain and CSF. An initial decrease in the release of dopamine is shown by the rapid fall in the ventricular CSF concentration of HVA. Histo-fluorescence studies revealed the accumulation of dopamine in the axons of substantia nigra, pars compacta neurons. Over a longer period of time, NMPTP appears to selectively and irreversibly damage neurons in the pars compacta of the substantia nigra, leading to chronic cell degeneration and ultimately to severe nerve cell loss and a marked reduction in the dopamine content of the striatum. Although diminished release of dopamine was evident in the lower levels of HVA in the ventricular CSF one day after the first dose of NMPTP, the monkeys failed to exhibit motor abnormalities until several doses of the drug had been administered. This is consistent with data showing that considerable reduction of dopamine and its metabolites may occur in parkinsonian patients without the development of clinical evidence of disordered function, presumably because of adequate compensatory activity in the surviving neurons. The pathological and biochemical changes produced by NMPTP are similar to the well established changes in patients with Parkinson's disease.

^a Significance: Information on the origin and kinetics of HVA in man will improve the clinical interpretation of HVA levels measured in body fluids and define their usefulness in describing disease states and monitoring the biochemical effects of therapeutic agents which act on brain dopamine systems. There is a need for an objective, biochemical test to measure the rate of brain dopamine turnover which can be used to: assess the severity of parkinsonism in the individual case; measure the progression of the disease; and, monitor the neurochemical effects of drug treatment.

Parallel studies in patients with idiopathic Parkinson's disease and in a good animal model of parkinsonism would allow one to study the mechanisms underlying the disease state and complications of L-dopa therapy, as well as to explore new therapies.

Proposed Course: In a continuing effort to estimate the central (brain) contribution to the total body production rate of HVA, the study of the HVA levels in CSF, blood, and urine will be extended to a group of medication-free patients with more severe parkinsonism (stage III-IV). The effect of different classes of antiparkinson drugs on HVA levels will also be examined.

Parallel studies will be carried out in patients with Parkinson's disease and in NMPTP-lesioned monkeys of: The role of changes in other neurotransmitter systems in the production of the clinical features of Parkinson's disease; (2) the qualitative, neurochemical differences between the stages of Parkinson's disease; (3) the pattern of metabolism, mechanism, and site of action of L-dopa in the damaged parkinsonian brain; (4) the neurochemical changes underlying L-dopa induced dyskinesia and of-off reactions; (5) the relationship between the neurochemical and motor effects of antiparkinson agents; and, (6) the therapeutic potential of relatively select D1 or D2 dopamine agonists.

Publications:

Burns, R.S., Calne, D.B.: The Disposition of Dopaminergic Ergot Compounds Following Oral Administration. In Calne, D.B., et al. (Eds.): Lisuride and Other Dopamine Agonists. New York, Raven Press, 1982.

Elchisak, M.A., Cosgrove, S.E., Ebert, M.H., Burns, R.S.: Distribution of Free and Conjugated Dopamine in Monkey Brain, Peripheral Tissues, and Cerebrospinal Fluid Determined by High Performance Liquid Chromatography. Brain Research. In Press.

LeWitt P.A., Burns, R.S., Calne, D.B.: Lisuride Treatment in Parkinson's Disease: Clinical and Pharmacokinetic Studies. In: Advances in Neurology, Vol. 37: Experimental Therapeutics of Movements Disorders. New York, Raven Press, 1983.

LeWitt, P.A., Miller, L.P., Newman, R.P., Burns, R.S., Insel, T., Levine, R.A., Lovenberg, W., Calne, D.B., Chase, T.N.: Tyrosine hydroxylase cofactor (tetrahydrobiopterin) in parkinsonism. Adv. in Neurol. In Press, 1983.

Burns, R.S., Gopinathan, G., Humpel, M., Dorow, R., and Calne, D.B.: The disposition of oral lisuride in patients with Parkinson's disease. Clinical Pharmacology and Therapeutics, In Press.

Weingartner, H., Burns, R.S., Diebel, R., LeWitt, P.A. The pseudodementia of Parkinson's disease: Distinguishing between effortful and automatic cognitive processes. Neuropsychologia, In Press.

Burns, R.S., Chiueh, C.C., Markey, S.P., Ebert, M.H., Jacobowitz, D.M., Kopin, I.J.: A primate model of Parkinson's disease: Selective destruction of substantia nigra, pars compacta dopaminergic neurons by N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. Proc. Natl. Acad. Sci. (USA). In Press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00153-06 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Treatment of Obsessional Children and Adolescents with Chlorimipramine		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH		
COOPERATING UNITS (if any) Clinical Neuropharmacology Branch, DCBR, NIMH National Institute of Neurological and Communicative Disorders and Stroke		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Child Psychiatry		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .25	PROFESSIONAL: .10	OTHER: .15
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Obsessional disorder of childhood</u> is a rare disorder which is severely disabling, and about which little is known. The purpose of this protocol is to collect basic data on family history of mental illness, <u>sleep measures</u> , CAT Scans and neuropsychological testing on a selected group of children and adolescents with severe primary obsessional disorder. A drug trial of <u>chlorimipramine</u> or placebo is being carried out to evaluate the effect of the <u>antidepressant</u> and the specificity of chlorimipramine for this disorder.		

Other Professional Personnel Engaged on Project:

Marcus Krusi, M. D. Clinical Associate, LCS, NIMH
 Martine Flament, M. D. Visiting Associate, LCS, NIMH
 Dennis L. Murphy, M. D., Chief, CNB, NIMH
 Theodore Zahn, Ph.D., Research Psychologist, LPP, NIMH
 Wallace Mendelson, M. D., Chief, Unit on Sleep Studies, CPB, NIMH
 Paul Fedio, Ph.D., Acting Chief, CN, NINCDS
 Martha Denckla, M. D. Chief, Section on Autism and Developmental
 Disabilities, DN, NINCDS

Objectives: To examine clinical, familial, physiological, neuropsychological and neuroradiological measures in childhood Obsessive-Compulsive Disorder. In addition, a double blind controlled trial of chlorimipramine is being conducted; drug response is related to the patients' clinical and biological characteristics.

Methods Employed: Patients are sought on a national level because of the rarity of the disorder. Inclusion criteria are Obsessive-Compulsive Disorder as a primary disturbance. Children must have IQ of 85 or above, and be free from known neurological disturbance or psychosis.

A modification of the Leyton Obsessional Inventory is used to monitor drug effects on Obsessive-Compulsive symptomatology throughout the 12-week clinical trial. Weekly ratings are made by two physicians and ward nurses on the CPRS.

Plasma levels of norepinephrine and of drug as well as platelet serotonin are being assayed at baseline and for each drug phase.

Major Findings: Twenty-four children have entered the protocol: eighteen males aged 13-17, and seven females aged 13-15. Baseline comparison of obsessive patients and age, sex and IQ matched controls indicates significant and specific deficits in visual-spatial functioning (Milner's stylus maze test and Money's road map test). In addition, obsessive patients had significantly higher ventricular brain ratios (VBRs) with planimetric examination. A variety of other subtle abnormalities in neurological examination (developmental immaturity in coordination and fine motor movements) and clinical EEG are also observed.

Preliminary data from the first 17 children who have completed the five-week chlorimipramine or placebo treatment trial indicates that chlorimipramine is superior, dramatically so for some children as measured by the modified Leyton Obsessional Inventory and the NIM Obsessive Rating Scale.

Clinical assessment of improvement is difficult in this group of patients owing to their secretiveness about rituals and the tendency for spontaneous remissions for periods lasting several months. The children are more labile in this respect than the adults participating in a parallel study.

Significance to Mental Health Research: Obsessive-Compulsive Disorder is a rare but extremely disabling condition. About one third of adults with the disorder had their onset during childhood or adolescence. Children with this condition are often very ill and 50% do not respond well to any treatment. Relative to even other rare conditions, such as infantile autism, there has been virtually no research in this area of childhood mental illness.

Propose Course of Project: A total of 30 patients will be studied. Baseline clinical measures, plasma tricyclic level, and platelet serotonin will be examined in relation to clinical response to active drug and to placebo. Neuropsychological and linguistic testing will be compared for patients and controls. A three-year follow-up is planned for all subjects.

Publications:

Rapoport, J.: Obsessive-Compulsive Disorder. In Shaffer, D., Ehrhardt, A., and Greenhill, L. (eds.): Diagnosis and Treatment in Pediatric Psychiatry, New York, McMillan, in press.

Berg, C., Behar, D., Zahn, T., Rapoport, J.: Obsessive Compulsive Disorder: an Anxiety Syndrome? In Gittleman, R. (ed.): Anxiety Disorders, Guildford Press, New York, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00161-05 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Behavioral Effects of Dietary Substances in Normal and Hyperactive Children		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Judith L. Rapoport, M.D., Chief, Section on Child Psychiatry, LCS, NIMH		
COOPERATING UNITS (if any) Laboratory of Psychology and Psychopathology, DCBR, NIMH Section on Experimental Therapeutics, LCS, DCBR, NIMH; U. of Fla., Gainesville, PMB, NICHD; U. of Maryland, Baltimore, Md.		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Child Psychiatry		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .40	PROFESSIONAL: .30	OTHER: .10
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Single doses of <u>caffeine</u> , in amounts within the diet of normal children, has been shown in previous studies to affect <u>vigilance</u> and <u>motor activity</u> . Currently, the subacute effects of caffeine are being examined with a group of 50 children selected for either high or low habitual <u>dietary caffeine intake</u> . The effects of two carbohydrates, <u>glucose</u> and <u>sucrose</u> , were compared with <u>(saccharin)</u> placebo in a group of boys considered to have adverse behavioral responses to <u>sugar</u> .		

Other Professionals Engaged in Project:

Marcus Krusi, M. D., Clinical Associate, LCS, NIMH
Ted Zahn, Ph.D., LPP, NIMH
Alan Neims, Ph.D., Professor of Pharmacology and Pediatrics, U. of
Florida, Gainesville, Florida
Marvin Cornblath, M. D., Professor of Pediatrics, University of
Maryland, Baltimore, Maryland

Objectives: Behavioral effect of caffeine in adults are best predicted by habitual dietary caffeine intake. The objectives of this study are to examine the relationship between dietary caffeine and behavioral response in prepubertal children.

The purpose of the sugar studies is to test the popular assertion that high sugar intake produces adverse behavioral effects in some normal and hyperactive children.

Methods Employed: A major feature of both of these studies is the effort that was made to select populations appropriate for diet testing. For the caffeine study, 800 grade school children completed a brief diet screening questionnaire. From this, a "high" caffeine consuming group ($> 10\text{mg/kg/day}$) was selected. A "low" caffeine consuming group ($\leq 1\text{mg/kg/day}$) was matched for age, sex and classroom. A total of 60 children were selected in this manner of whom 42 (mean age 9.5) agreed to participate in the challenge study. The seven-week outpatient study consisted of a baseline week, followed by two weeks of caffeine abstention. The final four weeks were a double blind crossover challenge with caffeine (5mg/kg twice a day) or placebo - each for two weeks. Dependent measures include classroom and home behavior, side effects, autonomic measures and a vigilance test. Compliance is monitored by salivary caffeine measurement (Dr. Neims). For the sugar study, families responded to a story in a local newspaper about our search for children considered "sugar reactive". Twenty-one children (mean age 10.4) were selected whose history was most suggestive that they became irritable, hyperactive and/or distractible following a high sugar load.

These children were tested on three separate days with behavioral tolerance tests. At 7:00 AM they received either glucose, sucrose or placebo (1.75 gram/kg). Motor activity, vigilance were rated over the following seven hours, and behavior was rated hourly.

Major Findings: Sugar challenge produced a slightly but significantly more rapid pulse over the course of the test day. With that exception, no change in vigilance or observer rated behavior was associated with sugar challenge. Motor activity was slightly but significantly decreased three hours following

sugar. Other physiological measures are still being examined. Blood glucose insulin and cortisol values, and shapes of GTT curves did not differ from that of a normal control population.

High caffeine consumers differed from low consumers at baseline in that they had more symptoms and anxiety and they were considered more restless and distractible by their teachers. The actual challenge study has not yet been completed, but preliminary findings indicate that high and low consumers are affected differently by caffeine. High consumers exhibit significantly fewer adverse effects.

Significance to Mental Health Research: In spite of wide public concern, there is almost no scientific data about dietary influence on behavior. Caffeine is widespread in the American diet. While caffeinism is an established clinical entity in adult psychiatry, it has not been described in children. It also important to investigate self selection of diets as this may provide clue for underlying physiological differences. Our data suggests that children who self select caffeine may have more "sluggish" physiological responses.

No adverse effects of sugar were demonstrated even in a population considered to be behaviorally sensitive to sugar. This underlines the importance of placebo control, and double blind technique in evaluating dietary influence on behavior.

Proposed Course of Project: Children are being sought with suspected unusual sensitivity to caffeine-containing beverages to evaluate possible cases of caffeinism and/or behavioral reactivity to caffeine.

The sugar challenge may be repeated with a preschool sample thought to be sugar reactive, and using parents as raters.

Publications:

Rumsey, J. and Rapoport, J.: Methodological Issues in the Study of Dietary Influence on Behavior in Pediatric Populations. In Wurtman, R. and Wurtman, J. (ed.): Advances in Nutrition Research, New York, Raven Press, in press.

Rapoport, J.: Methodology for assessing effects of Dietary Substance In Grade School Children. J. Psychiatr. Res., in press.

Several in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00162-04 LCS
PERIOD COVERED <u>October 1, 1982 to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Treatment of Hyperactive Children with Desmethylinipramine</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Judith L. Rapoport, M.D., Chief, Section on Child Psychiatry, LCS, NIMH</u>		
COOPERATING UNITS (if any) <u>Unit on Sleep Studies, CPB, IRP, NIMH</u> <u>Laboratory of Clinical Science, DCBR, IRP, NIMH</u>		
LAB/BRANCH <u>Laboratory of Clinical Science</u>		
SECTION <u>Section on Child Psychiatry</u>		
INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>.65</u>	PROFESSIONAL: <u>.40</u>	OTHER: <u>.25</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>A controlled trial of desmethylinipramine is comparing the acute and subacute (3 week) effects of this agent in hyperactive children, and relating the clinical effects to plasma concentration of the drug and to measures of norepinephrine metabolism.</u>		

Project Description:

Other Professional Personnel Engaged on Project:

Alan Zametkin, M.D. Clinical Associate, LCS, NIMH
William Z. Potter, M.D., Ph.D., Chief, Unit on Clinical
Psychopharmacology, CPB, IRP, NIMH

Objectives: As tricyclic antidepressants have been shown to have short-term beneficial effects for hyperactive children, this study is designed to examine these effects more closely. Desmethylinipramine (DMI) acts primarily by blocking reuptake of norepinephrine (NE). By monitoring plasma MHPG, NE, urinary NE, VMA and MHPG and examining clinical effects, changes in NE metabolism and anti-hyperactive effects could be examined in relation to plasma drug concentrations and the effect of drug on NE metabolism.

Methods Employed: DMI or placebo are being tried in a 3-week double-blind study; a total sample of 30 children is planned.

Major Findings: Twenty children have completed the study. DMI has a weak anti-hyperactive effect; somewhat less striking than that reported for IMI and for amitryptiline at other centers, and considerably less than that of stimulant drugs.

Significance for Mental Health Research: Hyperactivity is a major clinical problem in child psychiatry. Understanding its mechanism may lead to more effective treatment and possible prevention of disorders including alcoholism, sociopathy and schizophrenia.

Honors: Dr. Judith Rapoport received the 1983 Ittleson Award for Research in Pediatric Psychiatry from the American Psychiatric Association.

Projected Course of Project: The protocol will continue using DMI and placebo in a double-blind non-crossover design. The project has studied 20 children to date. However, the noncrossover nature of the study design necessitates larger numbers (at least 15 per group) in order for evaluation of relationships within the active drug treatment group. Of particular interest will be the change in urinary MHPG in relation to clinical change, as well as plasma concentration of the drug.

Publications:

Rapoport, J., Langer, D., and Ebert, M.: Pilot trial of mianserin treatment of hyperactive boys. In Greenhill, L. (Ed): Biological Aspects of Child Psychiatry. New York, Spectrum Publishers, 1982, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00163-04 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Naturalistic Study of Activity Levels of Hyperactive Children		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Linda Porrino, Ph.D., Guest Worker, Section on Child Psychiatry, LCS, NIMH		
COOPERATING UNITS (if any) Clinical Psychobiology Branch, DCBR, NIMH		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Child Psychiatry		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: .65	PROFESSIONAL: .40	OTHER: .25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Because of the availability of new technology for measuring <u>24 hour motor activity</u> , the NIH actometer, a study has been conducted to examine activity levels of <u>hyperactive children</u> and matched controls during a baseline week. Following this, motor activity of the hyperactive group was compared during <u>amphetamine</u> and placebo treatment periods. This is the first study to examine drug effects on motor activity outside of a laboratory setting. Drug effects were examined in relation to measures of structure of classroom and home environment. Actometer data was compared with traditional clinical measures for hyperactive children.		

Project Description:

Other Professional Personnel Engaged in Project:

Judith Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH
Thomas Wehr, M. D., Chief, Clinical Research Unit, CP, NIMH
Marcus Krusi, M. D., Clinical Associate, LCS, NIMH

Objectives: Hyperactive boys and matched controls who were in the same school, grade and classes were followed for a baseline week to compare 24 hour activity patterns for the groups. Following this, hyperactive boys were monitored continuously for four weeks; the effects of amphetamine (15 mg) or placebo were compared in a double-blind ABAB design. The point of the study was to see whether hyperactive children were more restless than controls (rather than more inattentive), in what situations, and to examine the effects of stimulant drugs.

Methods Employed: Motor Activity levels during school, free play and home activities were compared and related to measures of attention (Continuous Performance Test), and ratings of school and home structure. Children were monitored with the actometer worn on a belt which was worn even when they slept. Weekly appointments were kept at which time parent and teacher behavior ratings, side effects, hourly diaries of weekly activities and attentional measures were obtained.

Major Findings: A total of 12 patient-control pairs were studied. Findings indicate that hyperactive children are significantly more restless than controls even during sleep. Hyperactives are more active during a variety of activities - the group differences were most striking during school but were also significant after school and on weekends. Motor activity differentiated the groups better than did attentional tasks. Drug effects appear biphasic, decreasing activity during the day with some increase in activity in the evening. This "rebound" effect may represent altered receptor sensitivity and has not been reported elsewhere in clinical pharmacology.

This is the first clinical diagnostic study to use actometers as part of outpatient observation. They were powerful tools for possible differentiation of patient groups.

Significance to Mental Health Research: Only a naturalistic study such as this, can relate laboratory findings to clinically relevant situations. The nature of "hyperactivity" is poorly understood. Since hyperactive children are truly more restless than their peers, it is important to know for what situations this is true. Furthermore, this study provided data on behavioral "rebound" in the evenings following drug, indicating that for many children a multiple dose schedule is indicated.

This study has restored the credibility of the diagnostic label "hyperactivity" which had been removed as the core feature of the syndrome in DSM III renamed Attention Deficit Disorder. The study also had shed new

light on the qualities of the hyperactivity syndrome. For example, the increased motor activity during sleep indicates that some of the syndrome is relatively situation free. However, in comparison to controls hyperactives have particular difficulty in dampening down their activity level during the most structured school activities. Drug treatment does not lower activity below normal level but gives the children the ability to modulate their activity the way the controls can.

Proposed Course of Project: Further studies with different drug dose and schedules are planned.

Studies are planned to see if 24 hour motor activity differentiated conduct disordered children from those with primary diagnosis of Hyperactivity. Effects of dietary substances such as sugar and caffeine will be investigated in young conduct disordered and hyperactive boys.

Publications:

Rapoport, J.: Stimulant Drug Treatment of Hyperactivity: An Update, pp. 189-199. In S. Guze (ed.), Child Psychiatry - New Directions; New York, Raven Press, 1983.

Porrino, L., Rapoport, J., Ismond, D., Sceery, W., Behar, D., Bunney, W.: Twenty-four hour motor activity in hyperactive children and controls. Archives of General Psychiatry, in press.

Porrino, L., Rapoport, J., Ismond, W., Sceery, W., Behar, D., Bunney, W.: Twenty-four hour motoractivity of hyperactive children II; Effects of dextroamphetamine. Archives of General Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 00165-03 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological Markers of Alcoholism		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH		
COOPERATING UNITS (if any) Clinical Psychobiology Branch, NIMH Pregnancy Research Branch, NICHD		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Child Psychiatry		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 0.75	OTHER: 0.75
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The aim of the project was to measure putative biological markers for <u>alcoholism</u> in prepubertal boys who are <u>offspring of alcoholics</u> and in matched controls. Markers include: <u>blood and breath acetaldehyde</u> following a test dose of alcohol, and blood <u>alcohol and acetaldehyde dehydrogenase</u> , and transketolase in cell cultures from skin fibroblasts.		

Project Description:

Other Professionals Engaged On Project:

Martine Flament, M. D., Visiting Associate, LCS, NIMH
Markku Linnoila, M. D., Chief, Laboratory of Clinical Studies, DICBR,
NIAAA
Anil Mukherje, Chief, Section on Molecular and Developmental Genetics,
PRB, NICHD
Irwin J. Kopin, M. D., Chief, Laboratory of Clinical Science, NIMH

Objectives: There is evidence for a genetic factor particularly among male alcoholics. This pilot project compared blood and breath acetaldehyde for "high risk" and control children.

Schuckett had reported higher blood acetaldehyde for college age offspring of alcoholics compared with controls presumably matched for social drinking. Because of the difficulty of such a match, a comparison was made between prepubertal boys at risk for alcoholism and age matched controls.

Methods Employed: Extensive recruitment through area alcohol treatment programs has produced a sample of 11 high risk children and 11 age-matched controls. Clinical screening and skin biopsy were completed. A challenge of alcohol (0.5 ml/kg) followed by 4 hours of clinical and biological measures was completed.

Behavioral measures included memory test of Parker, et. al., the standing steadiness test for motor coordination, and mood scale of Shuckett and coworkers. All of these measures are sensitive to alcohol effects in adults and all were adapted for use with grade school children.

Blood samples for epinephrine, norepinephrine and cortisol were obtained at half hour intervals throughout the five hour test period.

Major Findings: Blood acetaldehyde and breath acetaldehyde and breath alcohol reached peak at 30 minutes, but did not differ between groups. Clinically, children did not become overly intoxicated in spite of moderate alcohol dose used. Plasma epinephrine increased significantly with alcohol, while plasma cortisol decreased. These physiological measures did not predict behavioral response to ethanol. In contrast, baseline mood state did predict behavior 30 minutes post alcohol ingestion. Children feeling ill at baseline tended to become tired and less talkative; children feeling tired or sad at baseline tended to become more lively.

Significance to Mental Health Research: As the treatment of alcoholism has been relatively unsatisfactory in adult samples, the identification and possible prevention of high risk individuals assumes great significance. Alcoholism in adults is a major public health problem.

Proposed Course of Project: The skin biopsies are being conducted in Dr. Mukherje's laboratory, NICHD. The alcohol and acetaldehyde dehydrogenase activities for the fibroblasts will be related to blood and breath acetaldehyde and to behavioral effects. In addition, transketolase activity is being measured as this has been reported higher in liver in alcoholics compared with controls (Blass, et. al.). Preliminary findings indicate transketolase may differentiate high risk and control fibroblasts.

Publications:

Behar, D., Berg, C., Linnoila, M., Wekon, W., Rapoport, J.: Behavioral and physiological effects of alcohol in high risk and control children.
Alcoholism: Clinical and Experimental Research. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00177-02 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Treatment of Hyperactive Children with Monoamine Oxidase Inhibitors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Judith L. Rapoport, M. D., Chief, Section on Child Psychiatry, LCS, NIMH		
COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS, NIMH Clinical Neuropharmacology Branch, NIMH Laboratory of Psychology and Psychopathology		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Section on Child Psychiatry		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.75	OTHER: 0.25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Hyperactive boys</u> are being treated with up to 15 mg/day of <u>clorgyline</u> , <u>parnate</u> or <u>deprenyl</u> (10 mg/day), selective and non-selective <u>Monoamine Oxidase Inhibitors</u> , or amphetamine (0.5/mg/kg). Behavioral measures include motor activity, vigilance and parent and teacher behavior ratings. Biological measures include urinary catecholamines and metabolites, urinary PEA and platelet MAO. The aim of the study is to elucidate neurotransmitter mechanisms mediating stimulant drug efficacy in hyperactivity.		

Project Description:Other Professionals Engaged on Project:

Alan Zametkin, M.D., Clinical Associate, LCS, NIMH
 Christy L. Ludlow, Ph.D., Speech Pathologist, DCP, NIMH
 Dennis Murphy, M. D., Chief, CN, NIMH
 Herbert Weingartner, Ph.D., Chief, Unit on Cognitive Studies, LPP, NIMH

Objectives: Reports of MAO inhibitors efficacy with a variety of childhood conditions prompted these clinical trials. The purposes of these studies was both to evaluate the efficacy of MAOIs for Attention Deficit Disorder with Hyperactivity, as well as to elucidate the neurotransmitter mechanisms in amphetamine treatment of hyperactivity.

Methods Employed: In the first study, six hyperactive boys were given amphetamine (0.5mg/kg) placebo and clorgyline (up to 15 mg) using a double blind crossover design modified by a two week placebo washout period between active drugs. Urinary catecholamines and metabolites and platelet MAO were measured to see if these predicted or reflected drug effects.

In the second and third studies, parnate (15 mg) and deprenyl (10 mg) are being used following the same study design. The purpose of these comparisons is to evaluate a selective MAO A inhibitor (clorgyline), a nonselective inhibitor (parnate) and a selective MAO B inhibitor (deprenyl) in the treatment of these children.

Major Findings: For the six children completing the first study, clorgyline appears to be almost as effective as amphetamine in reducing motor restlessness and improving attention span. There were no adverse reactions to clorgyline. The time course of these effects was slightly delayed compared to that of amphetamine, but more rapid than that for the antidepressant effect of clorgyline. Of considerable interest is the decrease in urinary MHPG seen with the clorgyline, which may show relationship to individual differences in clinical response. Platelet MAO did not change with either drug indicating that selective MAO A inhibition was achieved with clorgyline and that the MAOI strength of this dose of amphetamine was modest.

Ten children have completed the parnate study. Clinical impressions are that the drug is also efficacious and comparable to amphetamine in its clinical usefulness. There have been no adverse effects of the drug.

Significance to Mental Health Research: Hyperactivity for males, is a forerunner of adult sociopathy, alcoholism, and possibly schizophrenia. Studies on treatment and pathophysiology of hyperactive children have wide implications for prevention and treatment of these major conditions.

Proposed Course of Project: a trial of deprenyl is being started. Comparison of clinical efficacy of these MAOIs with that of amphetamine is being made, and the relationship to alterations in urinary catecholamines and metabolites will be examined.

Publications:

Three submitted

Several Abstracts published

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00178-02 LCS
PERIOD COVERED <u>October 1, 1982 to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Brain Structure and Function in Developmental Neuropsychiatric Disorders</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Judith M. Rumsey, Ph.D., Staff Fellow, Section on Child Psychiatry, LCS, NIMH</u>		
COOPERATING UNITS (if any) <u>Laboratory of Psychology and Psychopathology, NIMH</u> <u>Section on Brain Aging and Dementia, Laboratory of Neurosciences, NIA</u> <u>Section on Clinical Neuropsychiatry, NPB, NIMH</u>		
LAB/BRANCH <u>Laboratory of Clinical Science</u>		
SECTION <u>Section on Child Psychiatry</u>		
INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>1.5</u>	PROFESSIONAL: <u>1.0</u>	OTHER: <u>0.5</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Children and adults with pervasive developmental disorder (PDD), including autism, have been studied with <u>brainstem auditory evoked potentials</u>. Results indicate prolonged neural transmission times are <u>not</u> characteristic of PDD. Other abnormalities seen may be attributable to peripheral audiological factors rather than to brainstem pathology.</p> <p><u>Adults</u> with childhood diagnoses of autism are in addition, being studied with <u>CT scans, PET scans, neuropsychological testing, psychiatric interviews</u>, and supplementary measures. Results on eight autistic adults and 12 controls suggest normal brain anatomy, diffusely elevated rates of cerebral glucose utilization, and no regions of deficient glucose use. Clinical outcomes include an absence of positive schizophrenic symptoms, a high incidence of concrete and obsessional thinking, compulsive, stereotyped routines, motor stereotypies, deficits in abstract problem-solving, as measured by "frontal lobe" testing, and impairments in social-adaptive functioning exceeding IQ-based expectations. Matched normal and schizophrenic controls are being tested with neuropsychological measures.</p> <p>New projects include <u>cortical evoked potentials, spectral analysis and topographic mapping of EEG, and cerebral blood flow studies of language-disordered dyslexic, visuospatially-disordered dyscalculic, and attention deficit-disordered children and dyslexic and autistic adults</u>. These studies will combine physiological measurement with neuropsychological activation tasks to identify abnormalities in temporal information-processing and to localize dysfunction.</p>		

Project Description:**Other Professionals Engaged on Project:**

Judith L. Rapoport, M.D., Chief, Section on Child Psychiatry, LCS, NIMH
 Connie Duncan-Johnson, Ph.D., Psychologist, LPP, NIMH
 Richard Coppola, Ph.D., Engineer, LPP, NIMH
 Stanley I. Rapoport, M. D., Chief, LN, NIA
 Ronald Zec, Ph.D., Psychologist, NPB, NIMH
 Anita Pikus, M. S., Audiologist, CC, NIH
 Daniel Weinberger, M. D., NPB, NIMH

Objectives: Ongoing and new studies are aimed at identifying neuroanatomical, neurophysiological and neuropsychological processing deficits which characterize autism, attention deficit disorders, and specific subtypes of learning disabilities. Neurophysiological procedures should prove valuable both in localizing dysfunction and in providing information on "where" information processing is breaking down in a temporal sense (e.g., attentional stage, sensory-perceptual processing, cognitive decision-making). Another objective is the study of outcomes in autism and dyslexia.

Methods: Methods include CT scans, PET scans, EEG spectral analysis combined with topographic mapping, brainstem and cortical evoked potentials, measurement of regional cerebral blood flow using xenon inhalation, neuropsychological testing, psychiatric interviews, behavioral questionnaires, and supplementary measures.

Major Findings: A recently-completed study of brainstem auditory evoked potentials in children and adults with pervasive developmental disorders, including autism, indicates that when careful attention is given to technical factors, little evidence of prolonged neural transmission times is seen, in contrast to earlier, poorly-controlled studies. Technical factors include the establishment of high reliability of evoked potential recordings, blinded independent evaluation of recordings for wave identification, matching of normal controls by sex and age, and independent evaluation of audiological status, all of which were given careful attention in our study.

Other abnormalities seen in certain patients, i.e., shortened transmission times, may reflect frequency-specific hearing losses. Our results, in conjunction with recently published data from intracerebral recordings in humans, suggest that abnormalities seen in our study may be attributable to more peripheral portions of the auditory pathway than previously thought.

CT scans of autistic adults and age- and sex-matched normal controls are being analyzed with a computerized, quantitative technique. Thus far, results suggest normal ventricular size in all but one autistic patient. Ventricular asymmetries and asymmetries of brain tissue are in the process of being measured.

PET scan results on eight autistic men and 12 age- and sex-matched controls suggest that diffusely elevated rates of glucose use are associated with autism. No regions of deficient glucose use and no hemispheric asymmetries are seen in our autistic patients.

An Institute on Aging study of young men with Downs syndrome shows even more highly elevated rates in the small number of patients studied to date. This raises the possibility that an inefficient use of glucose may characterize various developmental disorders in which neuropathology is nonobvious.

This is the first systematic follow-up study of adults with childhood diagnoses of autism. Psychiatric and behavioral studies of 12 autistic adults indicate continuing residual symptoms of autism and an absence of positive schizophrenic symptoms (hallucinations and delusions). Concrete and obsessional thinking, stereotyped, compulsive routines, and motor stereotypies are prevalent among this sample. The presence, nature and severity of language disorder varies considerably. Social-adaptive functioning falls below expectancies based on IQs.

Neuropsychological testing, thus far, documents a high prevalence of relatively superior visuospatial skills and impairments in abstract nonverbal problem-solving, as measured by "frontal lobe" testing. Normal controls matched for education, IQ, sex and age are being tested to ascertain other specific deficits (e.g., memory deficits). A schizophrenic contrast group is also being tested.

Significance to Mental Health Research: PET-scan findings suggest the possibility of some diffuse dysfunction that results in an inefficient use of glucose throughout the brain. Results thus far fail to support brainstem dysfunction in autism. Neuropsychological testing would suggest a more localized picture of neurological dysfunction than do our PET scan. Psychiatric and behavioral studies suggest continuing impairments in psychiatric, cognitive, and social functioning even in the presence of average or higher IQs and suggest that autism is distinct from adult psychoses.

Proposed Course of Project: Papers on the results of some of these studies (brainstem auditory evoked potentials in pervasive developmental disorders, PET-scan findings in autistic adults) are in progress. Additional autistic adults are being studied. CT-scan results are being further analyzed. Normal and schizophrenic contrast groups are being tested with neuropsychological measures.

Cerebral blood flow and electrophysiological studies of learning disabled children and adults, attention deficit disordered children, and autistic adults are in the mid-to-late planning stage. We hope to begin screening children for these projects this summer and testing children with some electrophysiological techniques possibly late in summer, 1983.

Publications:

Rapoport, J., Ismond, D. Biological research in child psychiatry: Effects on Practice and Theory. Journal of the American Academy of Child Psychiatry, 1982, 21:543-548.

Rapoport, J. L., Rumsey, J., Duara, R., Schwartz, M., Kessler, R., Cutler, N. & Rapoport, S. I. Cerebral metabolic rate for glucose in adult autism as measured with position emission tomography (PET). Proceedings of the 6th International Meeting of Cerebral Blood Flow, Paris, June, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00301-01 LCS
PERIOD COVERED <u>October 1, 1982 to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Diagnosis in Child Psychiatry</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Judith L. Rapoport, M.D., Chief, Section on Child Psychiatry, LCS, NIMH</u>		
COOPERATING UNITS (if any) <u>Department of Psychiatry, The Maudsley Hospital London, The Grant Foundation, New York, N.Y.</u>		
LAB/BRANCH <u>Laboratory of Clinical Science</u>		
SECTION <u>Section on Child Psychiatry</u>		
INSTITUTE AND LOCATION <u>NIMH, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>.65</u>	PROFESSIONAL: <u>.30</u>	OTHER: <u>.35</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>A cross national study of the diagnosis of childhood hyperkinesis is being conducted between the U.S. (NIMH) and the U.K. (The Maudsley Hospital) to understand the basis for the widely discrepant rates of diagnosis between the two countries. In the U.S. Hyperkinesis accounts for nearly 50% of child guidance clinic cases, while in the U.K. this diagnosis accounts for less than 3%.</u>		

Project Description:**Other Professional Personnel Engaged on Project:**

Maureen Donnelly, M.D., Clinical Associate, LCS, NIMH
Alan J. Zametkin, M.D., Clinical Associate, LCS, NIMH
Eric Taylor, M.D., Senior Registrar, The Maudsley Hospital, London
Michael Pendergast, M.D., Registrar, The Maudsley Hospital, London
Michael Rutter, M.D., Professor of Child Psychiatry, The Maudsley Hospital, London

Objectives: To examine the differences in diagnostic practice between the U.S. and U.K. particularly with reference to the diagnosis of hyperkinesis. Differences between ICD-9, the current European system, and DSM III the current American diagnostic system will be explored.

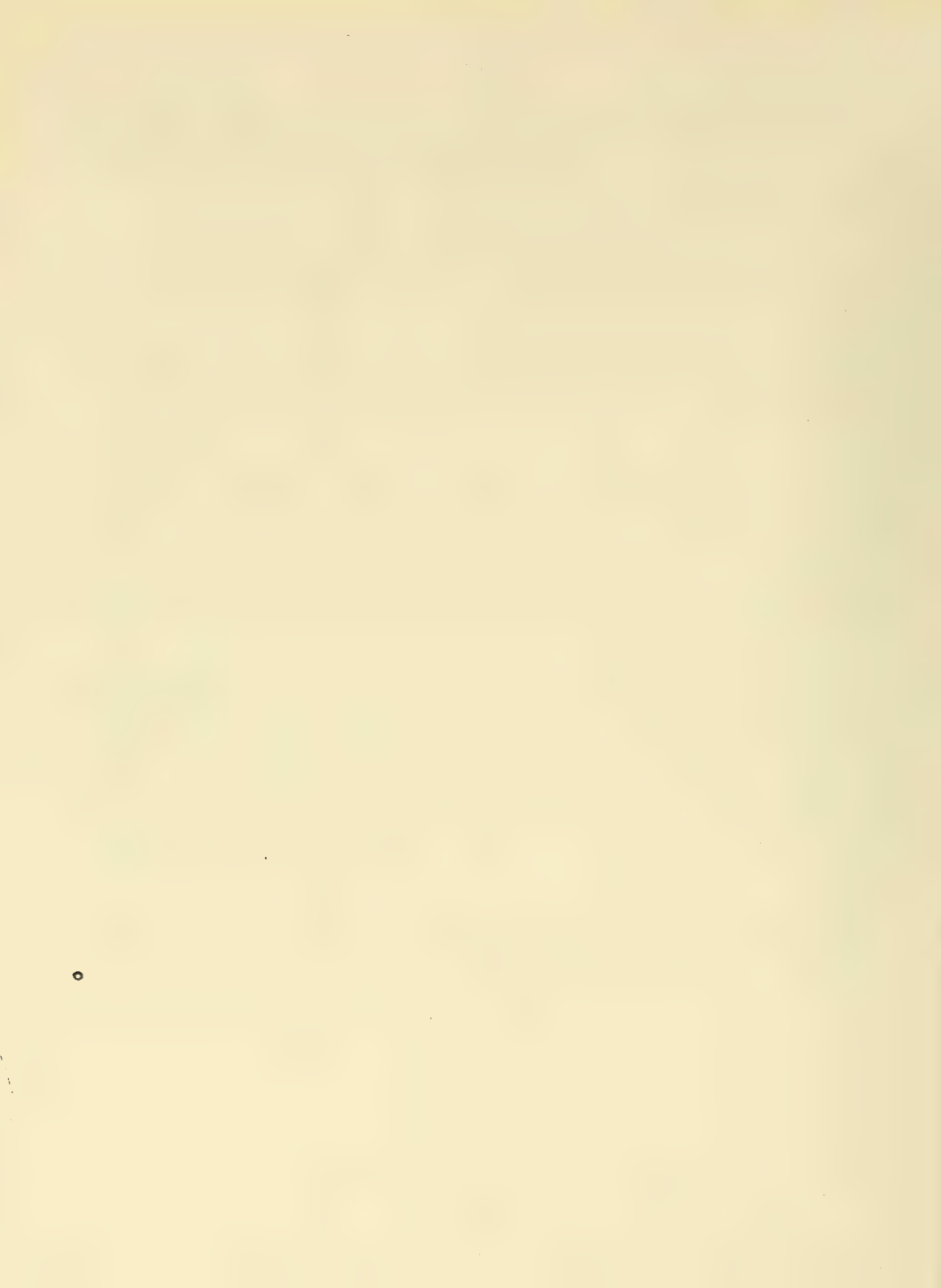
Method Employed: Patients referred to the Division of Child Psychiatry of the Maudsley Hospital in London, and to the Section on Child Psychiatry, NIMH are being evaluated in a systematic manner. Initial assessment includes a semistructured videotaped interview, standardized interviews with parents and children and psychometric testing. Cases will be diagnosed by research teams at the two hospitals and by local clinicians and academic child psychiatry. Two diagnostic systems will be used - the DSM III and ICD-9. Tapes and histories will be exchanged between the two centers. A pilot study using 40 taped case history exchange will precede the larger study which will involve more complete follow-up information from the two centers and larger samples.

Major Findings: A total of 20 cases have been assembled at each center. Preliminary exchange of tapes and rating indicates that much of the diagnostic discrepancy between the centers is accounted for by the wide use of the ICD-9 category of Conduct Disorder, defined more broadly than that of DSM III. Furthermore, the British make wide use of "Mixed Disturbance of Emotions and Conduct" while DSM III forces clinicians to choose between neurotic disorders and conduct disorders. Finally, the lack of interest in stimulant drug treatment may predispose British clinicians to ignore the specific symptoms of restlessness and inattentiveness.

Significance to Mental Health Research: It is of considerable interest to understand the wide variation in rates for childhood hyperactivity across different countries. While it is possible that different genetics, social structure or even environmental toxins might be responsible for such differences it is essential that similar methodology be employed in order for any true differences to even be established.

Proposed Course of Project: Tapes will be rated by local clinicians and by research teams over the following year. Follow-up studies will compare the predictive validity of both systems with both the U.S. and U.K. populations.

Publications: None to date.



DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00271-14 LCS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Fate of 3-Methoxy-4-Hydroxy-Phenyl Glycol in Primates		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Irwin J. Kopin, Chief, LCS LCS NIMH		
COOPERATING UNITS (if any) Section on Experimental Therapeutics, LCS		
LAB/BRANCH Laboratory of Clinical Science		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Gas liquid chromatography-mass spectrometry (GLC-MS) and high pressure liquid chromatography (HPLC) are used to isolate and measure homovanillic acid (HVA), 3-methoxy, 4-hydroxyphenylglycol (MHPG) and vanillyl mandelic acid (VMA) and other catecholamine metabolites in the urine of controls and patients with orthostatic hypotension as well as with various neurologic and mental disorders.</p> <p>Deuterated D(-) MHPG has been injected intravenously into human controls quickly and as a slow infusion. Blood and plasma values for endogenous and deuterated MHPG and VMA have been analyzed to study the kinetics of conversion of MHPG to VMA and the rates of elimination of the compounds and CSF examined to determine penetration into this fluid compartment. The deuterated MHPG also equilibrates with brain, accounting in part for the covariance of regional MHPG levels in different areas of brain.</p>		

Other Professional Personnel Engaged on Project:

Michael H. Ebert	Chief, Section on Experimental Therapeutics	LCS NIMH
Edna K. Gordon	Chemist	LCS NIMH
Michael H. Ebert	Chief, Section on Experimental Therapeutics	LCS NIMH
Jerry A. Oliver	Chemist	LCS NIMH
Robert L. Sherman	Biologist	LCS NIMH
Sanford P. Markey	Chief, Section on Analytical Biochemistry	LCS NIMH
David Jimerson	Staff Physician	LCS NIMH

Objectives: Determine the distribution and metabolic fate of MHPG in body fluids to understand the physiological significance of changes in levels of the norepinephrine metabolite.

Methods Employed: Deuterated MHPG injected as a bolus or administered as a slow infusion over 3-4 hours is excreted in urine as a conjugate of MHPG or converted to VMA. Kinetic analysis of plasma free MHPG indicates that unconjugated plasma MHPG is a major transitional metabolite which accounts for about 2/3 of the total urinary catecholamine metabolites. About half of the MHPG in plasma is excreted as a conjugate whereas the remainder is converted to VMA and accounts for about half of the VMA excreted in the urine. Both conjugated MHPG and VMA are derived from sources independent of plasma MHPG e.g., DHPG (Drs. Ebert, Markey, Blombery, Mrs. Gordon, Mr. Oliver and Dr. Kopin).

Major Findings: Although there is a popular concept that total urinary MHPG may reflect brain catecholamine metabolism, we have obtained good evidence which indicates that less than 30% of urinary MHPG is derived from brain.

Plasma MHPG levels may however provide a useful index of cumulative sympathetic activity in the whole body. The levels of MHPG in plasma are significantly greater in depressed patients who fail to suppress plasma cortisol levels in response to dexamethasone than in patients who show normal suppressions (Drs. Jimerson, Insel, Reus, and Kopin). These results suggest that in a subgroup of depressed patients there may be hyper-reactivity of both the hypothalamic-pituitary-adrenal cortisol and sympathetic responses to illness-related stress.

CSF obtained at varying intervals after initiation of a constant fusion of deuterated MHPG contains the labelled compound at concentrations which approach those in plasma by 4-8 hours, indicating that there is an exchange of plasma and CSF MHPG (Drs. Ebert, Kopin, Mrs. Gordon and Mr. Oliver). This is consistent with the observation that plasma and CSF levels of free MHPG are highly correlated. CSF levels are always higher than those in plasma, even when large amounts of the catecholamine metabolite are derived from the tumor of the adrenal medulla. This is explained by a plasma and CSF two-compartment system with similar rate constants for entry into and exit from the CSF compartment. MHPG formed, but not metabolized, in the central nervous system maintains CSF levels of MHPG at a constant increment over those in plasma. Estimation of this increment provides the best available index of formation of MHPG in the central nervous system (Drs. Kopin, Polinsky, Mrs. Gordon and Dr. Jimerson). In monkeys deuterated MHPG enters brain from plasma and the exchange of plasma with brain MHPG explains, at least in part, the covariance of MHPG levels in various areas of brain (Dr. Kopin, Mr. Oliver, Mr. Sherman, Drs. Burns and Markey).

Significance to Biomedical Research and the Program of the Institute:

MHPG is a major catecholamine metabolite, particularly in brain and CSF. Its rate of excretion has been claimed (incorrectly) to reflect brain adrenergic activity. These studies are aimed at defining the usefulness of MHPG levels and excretion as an index of adrenergic activity for use in clinical studies of neuro-psychiatric disorders and effects of drugs used to treat these disorders.

Honors: Dr. Irwin J. Kopin was awarded First Prize for 1983 by the Anna Monika Foundation for work on the disposition and metabolism of MHPG.

Proposed Course: Continued studies of MHPG plasma CSF levels and urinary excretion in disease states and during drug administration.

Publications:

Kopin, I.J.: Evolving views of the metabolic fate of norepinephrine. *Endocrinologia Experimentalis* 16: 291-300, 1982.

Kopin, I.J., Gordon, E.K., Jimerson, D.C. and Polinsky, R.J.: Relation between plasma and cerebrospinal fluid levels of 3-methoxy-4-hydroxyphenylglycol. *Science* 219: 73-75, 1983.

Jimerson, D.C., Insel, T.R., Reus, V.I. and Kopin, I.J.: Increased plasma MHPG in dexamethasone-resistant depressed patients. *Arch. Gen. Psychiatry* 40: 173-176, 1983.

Kopin, I.J., Blombery, P., Ebert, M.H., Gordon, E.K., Jimerson, D.C., Markey, S. P. and Polinsky, R.J.: Disposition and metabolism of MHPG-CD₃ in humans: Plasma MHPG as the principal pathway of norepinephrine metabolism and as an important determinant of CSF levels of MHPG. Paper presented at Skokloster, Sweden, 1982, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00247-08 LDP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Patterns of Psychological Functioning in Children with Endocrine Abnormalities

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jerome H. Blue

Staff Fellow

LDP

NIMH

COOPERATING UNITS (if any)

University of Vermont School of Medicine, Burlington, Vermont

University of Pittsburgh, Pittsburgh, Pennsylvania

National Naval Medical Center, Bethesda, Maryland

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00257-07 LDP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Effects of CNS Treatment on Intellectual Functioning of Children with Leukemia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Howard A. Moss

Guest Worker

LDP NIMH

COOPERATING UNITS (if any)

Various Children's Hospitals throughout the United States
National Cancer Institute

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years:

.40

PROFESSIONAL:

.15

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research is based on previously obtained findings that CNS treatment of patients with acute lymphocytic leukemia (ALL) had the delayed effect of producing a deficit in intellectual functioning that appears to be somewhat progressive and is greater among children treated at a younger age. The current research involves comparing children who receive either the standard CNS treatment or an alternative non-CNS treatment. ALL patients are tested on a series of neuropsychological measures dealing with short and long term memory, attention, perceptual-motor functioning, coordination, concept formation, academic skills, and general cognitive functioning. These children are studied longitudinally and are assessed several times over a five to seven year period. The purpose of the assessments is to determine if the non-CNS treatment is less neurotoxic and results in more or less neuropsychological deficits than the standard CNS treatment.

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project:

David G. Poplack

Senior Investigator

POB NCI

Project Description:

The chance of survival from acute lymphocytic leukemia (ALL) has increased over the past 15 years because of the utilization of new treatment procedures. Whereas the survival rate prior to these changes was minimal the current survival rate ranges between 50 to 80 percent, dependent on the risk status at the time of diagnosis (age of child and initial white blood cell count). The earlier treatment consisted of intravenous chemotherapy, which usually followed a course of remission and then almost inevitable relapses because of leukemic cells that found sanctuary in the central nervous system. The leukemic cells that remained in the central nervous system were protected from the intravenous chemotherapy by the blood-brain barrier. The more current and considerably more successful approach for treating ALL involves direct treatment of the central nervous system (CNS) through the administration of a series of cranial irradiations (usually 2400 rads) and directly injecting powerful anti-cancer drugs into the central nervous system (usually intrathecal methotrexate).

Initially, this CNS treatment was considered to be minimally neurotoxic with few adverse side effects because mature (brain) cells are considered to be non-radiosensitive. This thinking has gradually changed because of increased observations of learning and developmental problems and because of an accumulation of physical evidence of brain changes among children receiving this CNS treatment. Because of the concern over possible adverse effects we conducted a retrospective study of a group of children who had undergone CNS treatment and survived ALL for a period of 3-7 years. Their intellectual functioning was compared to that of matched siblings. It was found that these children obtained IQ's about 15 points lower than their siblings. Moreover, the younger the child, the longer the interval since treatment, and the brighter the sibling, the greater the deficit.

As an outgrowth over the increasing concern about the neurotoxicity and progressive, deleterious effects of the CNS treatment, a new medical protocol has been initiated. This protocol compares the therapeutic results of this form of treatment with a newly developed treatment (high dosage intravenous methotrexate infusion) to determine if this new treatment is equally efficacious but less neurotoxic than the traditional CNS treatment. The psychological component of this research consists of administering a specially devised battery of neuropsychological procedures and behavioral measures to patients and to control cases to determine if there are differential effects on various psychological functions from these two forms of treatment.

A clinical study under the direction of the National Cancer Institute includes patients from five hospitals across the country. The purpose is to compare the effectiveness and the degree of neurotoxicity associated with treating acute lymphoblastic leukemia (ALL) patients (a) with cranial irradiation plus intrathecal methotrexate or (b) with high dosage systemic methotrexate infusions. It is known that cranial irradiation and intrathecal methotrexate produce structural changes in the brain and we have also established that this treatment results in lowered intellectual functioning. It is unknown what effects the high dosage infusion methotrexate has on the central nervous system. The

sample will consist of children from 1 to 21 years of age who will be evaluated annually for a five- to seven-year-period. The first assessment will be made after remission is brought about from standard chemotherapy but prior to initiation of the central nervous system or high dosage infusion methotrexate treatment. The battery of assessments includes: the Stanford-Binet, McCarthy, or Wechsler scales, in terms of their age appropriateness, for measuring intellectual functioning, a series of neuropsychological procedures for measuring attention, new learning, problem solving, immediate and delayed memory, academic achievement, and sensory and tactile motor functioning (assessments of these specific abilities will be helpful in identifying the localization of brain impairment), the Achenbach Behavior Checklist will be used for measuring social competence and behavioral problems, and a modification of the Block Child Personality Q-Sort procedure (administered to the mothers) to determine if personality characteristics tend to be altered. This research includes two control groups: a group of matched siblings and a group of children with solid tumors who do not receive any treatment directed at the central nervous system. These control groups will be tested twice at a yearly interval and will be selected so as to be representative of the patient sample in terms of age, ordinal position, and socioeconomic status.

Significance to Biomedical Research:

The focus of this research is on the behavioral effects of different medical treatments. The findings from prior research, on a sample of long-term survivors, influenced the search for an alternative treatment for ALL which is as effective as CNS treatment, in allaying the disease, but less neurotoxic. This research monitors the effects of the respective treatments for a variety of psychological functions. Such information is relevant also for planning remedial programs for children who have experienced these treatments. Information we have obtained has been used to counsel parents and school personnel as to a child's status and potential, and in special cases recommend placements. As a practical aspect of this research the Pediatric Oncology Branch of NCI has initiated a follow-up clinic to evaluate behavioral difficulties deriving from the treatment and make recommendations aimed at facilitating the child's adjustment.

Proposed Course:

About 150 children have been included in the study and several have received their second and third evaluations. Preparations are being made to evaluate sibling controls. Since the possible behavioral effects of the CNS treatment appear to be delayed it will be a few years before meaningful analyses can be carried out.

Publications:

Kellerman, J., Siegel, S., and Moss, H. A.: WISC-R Verbal Performance discrepancy in children with cancer: A statistical quirk. J. Ped. Psychol., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02135-06 LDP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Emotional Development in Children of Bipolar Depressed and Normal Parents

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Carolyn Zahn-Waxler Research Psychologist

LDP NIMH

COOPERATING UNITS (if any)

Laboratory of Biological Psychiatry, NIMH

Laboratory of Psychobiology, NIMH

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL Staff Years

1.15

PROFESSIONAL:

.90

OTHER:

.25

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This research is concerned with the psychological development of young children from families in which one parent has a history of bipolar affective illness and children from families in which there is no diagnosed parental psychopathology. Children are studied longitudinally beginning at 12 months of age. Observations in the home, mothers' reports and a series of laboratory sessions provide assessments of children's affective and interpersonal functioning. Children from bipolar families were more likely to have behavior problems, disturbances in attachment patterns, deficits in social-cognitive functioning, and difficulties in interaction with peers. These children also showed considerable dysregulation of emotion in stressful situations. Childrearing practices differed in normal and bipolar families.

Names, titles, laboratory and institute affiliation of other professional personnel engaged on the project:

Marian Radke-Yarrow	Chief	LDP NIMH
Mark Cummings	Staff Fellow	LDP NIMH
Ronald Iannotti	Research Psychiatrist	LDP NIMH
Leon Cytryn	Research Psychiatrist	LDP NIMH
Donald McKnew	Research Psychiatrist	LDP NIMH
Yolande Davenport	Social Worker	CPB NIMH

Project Description:

The focus of this research is on the psychological development of children from normal families and children from families in which one parent has a pervasive and enduring affective disturbance (bipolar affective disorder). Children were studied longitudinally beginning at 12 months of age. Observations in the home, mothers' reports and a series of laboratory sessions provide assessments of children's affective and interpersonal functioning. Children from bipolar families were more likely to have behavior problems, disturbances in attachment patterns, deficits in social-cognitive functioning, and difficulties in interaction with peers. These children also showed considerable dysregulation of emotion in stressful situations. The design and findings are described in greater detail in annual report Z01-MH 02135-05.

Significance to Biomedical Research:

There is substantial evidence of genetic transmission of manic-depressive illness. Transmission of the illness across generations, however, may also be influenced by the disordered emotions and environments in which children of manic-depressives are reared. This research identifies some of the deviant child-rearing practices used in bipolar families and the emotional problems that may result for the children as early as the first years of life. This information is directly relevant in planning prevention and intervention strategies in families with severe emotional problems: the aim is to decrease the likelihood that future generations of children will be affected by the affective disorders of their parents.

Proposed Course:

This is a final report. Manuscripts accepted for publication are listed below

Publications:

Zahn-Waxler, C.: Maternal child rearing practices in relation to children's altruism and conscience development. In Hare, A. P., Blumberg, H. H., Kent V. and Davies, M. (Eds.), Small Groups: Social Psychological Processes, Social Action and Living Together. London, John Wiley & Sons, 1983, Vol. 1,

Zahn-Waxler, C., McKnew, D. H., Cummings, E. M., Davenport, Y. B., and Radke-Yarrow, M.: Problem behaviors and peer interactions of young children. Am. J. of Psychiatry, in press.

Zahn-Waxler, C., Cummings, E. M., McKnew, D. H., and Radke-Yarrow, M.: Affective arousal and social interactions in young children of manic-depressive parents. Child Dev., for special issue on psychopathology, in press.

Davenport, Y. B., Zahn-Waxler, C., Adland, M. L., and Mayfield, A.: Early child rearing practices in bipolar families. Am. J. Psychiatry, in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02142-05

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Behavioral Studies of Children with Juvenile Diabetes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Howard A. Moss

Guest Worker

LDP NIMH

COOPERATING UNITS (if any)

Local Community Physicians in Private Practice

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☒ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project has been discontinued.

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Leon Kuczynski	Visiting Associate	LDP	NIMH
Sarah Friedman	Research Psychologist	LDP	NIMH
Barbara Hollenbeck	Research Social Science Analyst	LDP	NIMH
Judy Stilwell	Social Science Analyst	LDP	NIMH

Project Description:

The objective of this research is to develop a paradigm by which the childrearing environment can be investigated in detail. Typical rearing functions and parent and child behavior are kept intact by having the participants create their own environment in the laboratory. This is done by having mother and children come to the laboratory, an informal homelike apartment, for a series of half-days. Much of the structure and many of the events in these visits are of the participant's own making. Conditions allow and encourage usual routines, behavior, and emotions (eating, playing, learning, disciplining, resting, being happy, angry, upset, etc.). There is also an underlying experimental structure in the sessions; standard events (e.g. mother leaves temporarily, mother is made busy in ways that take her attention from the child, a visitor arrives) are introduced as naturally as possible in order to elicit certain classes of response. The procedures, with appropriate changes geared to the age of the children, are repeated at intervals of 1-1/2 years to permit longitudinal study. Normal families and families with psychopathology are the participants. Parents and two siblings (initial ages 2 years and 5 to 8 years) are studied.

There are a number of reasons for developing a sensitive instrument by which to assess the rearing environment in a controlled way and to observe behavior of parent and child in that environment: (a) Progress in understanding interaction between genetic or constitutional factors and environmental factors necessitates assessments of learning conditions in terms and in ways that identify and measure variables with a level of precision that is not achievable in global ratings, verbal reports, and brief experimental procedures. (b) A method by which the rearing environment is made accessible for detailed study is crucial for identifying the processes of transmission of adaptive and maladaptive behavioral patterns.

The sessions in the apartment vary in the family composition: Mother is seen with each child alone and with children together; the siblings are seen together and with peers; the father is seen with the family. In addition to the observed sessions, further data on parental beliefs, practices, and support systems are obtained through observations in the homes and by interview and paper-and-pencil instruments. Parent and child behaviors are coded "live" and from audiovideo tapes.

This research strategy is directed to two parallel substantive objectives: To study normal rearing and behavioral developmental processes, and to investigate the nature of functional impairment of depressed mothers (bipolar, major unipolar, or minor depression) and the functioning of their children. Research on the latter objective, of course, requires the normal comparison group.

This paradigm is the basis of the studies reported in Z01 MH 02152, Z01 MH 02156. Dimensions of rearing that are investigated include the cognitive content of rearing, teaching functions, regulation and control, affective communications. Children's affective qualities, cognitive functioning, and social interaction, and psychiatric status are assessed. This paradigm is also the source of data for investigating a number of methodological issues.

One important methodological problem in research in child development concerns the influences of varying behavior settings on behavior. A recurrent finding is that behavior in one situation is not predictive of the same behavior in other situations. The issue is often ignored in research by limiting data collection to single constrained settings or by summing across situations. An understanding of situational determinants of behaviors is needed (a) to evaluate individuals' abilities to adapt to changing requirements of the situation, (b) to understand the kinds of behaviors that are facilitated and inhibited by different contexts, and (c) to determine which behaviors are particularly sensitive to situation and which are stable across situations. The present study investigates the consequences for research findings of selecting given settings for sampling parent and child behaviors.

Significance to Biomedical Research:

In order to evaluate environmental contributions to children's development, sound measures of the environment are needed which go well beyond global categories of assessment and verbal reports. Conceptualization and measurement of environment have been seriously deficient in research on genetic-environment interactions and on specific environmental contributions to children's behavior problems and psychiatric disorders. In this study, an effective research method has been developed.

Proposed Course:

This is a longitudinal study in which families are followed over a period of 3 years. The sample now consists of 80 families. Families in particular diagnostic categories must still be recruited. It is anticipated that 3 to 4 years will be required for completion of data collection.

Publications:

Barrett, D.E., Radke-Yarrow, M. and Klein, R.E.: Chronic malnutrition and child behavior: Effects of early caloric supplementation on social and emotional functioning at school age. Dev. Psychol. 18: 541-556, 1982. Reprinted in: Chess, S. and Thomas A. (Eds.): Annual Progress in Child Psychiatry. New York, Brunner/Mazel, 1983, vol. 16.

Yarrow, M.R. and Kuczynski, L.: Conceptions of environment in child rearing interaction. In Magnusson, D. and Allen, V. (Eds.): Personality Development as Person-Environment Interaction. New York, Academic Press, 1983.

Yarrow, M.R. and Waxler, C.Z.: Roots, motives and patterning in children's prosocial behavior. In Reykowski, J., Karylowski, J., Bar-Tal, D., and Staub, E. (Eds.): The Development and Maintenance of Prosocial Behavior: International Perspectives. New York, Plenum Press, in press.

Yarrow, M.R., Waxler, C.Z., and Chapman, M.: Children's prosocial dispositions and behavior. In Mussen, P.H. (Ed.): Manual of Child Psychology (Vol. IV, 4th ed.). New York, John Wiley & Sons, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02146-04 LDP
PERIOD COVERED October 1, 1981 through September 30, 1982		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Etiology of Problem Aggression in Early Childhood		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> <div style="display: flex; justify-content: space-between; padding: 0 10px;"> E. Mark Cummings Staff Fellow LDP NIMH </div>		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years: 1.65	PROFESSIONAL: 1.05	OTHER: .60
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; padding: 0 10px;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This research focuses on the <u>nature and etiology of problem aggression</u> in young children. In one study the characteristics of children's <u>aggression in the home</u> are assessed between 1 and 2 1/2 years of age. In a second study specific hypotheses concerning <u>situational, familial, and dispositional determinants of aggression</u> are tested on the basis of a series of laboratory procedures administered at two and again at five years of age. Also planned is a third study focusing on the aggression of very young children who have been selected because of problem aggression. The early existence of stable patterns of aggression among young boys was found. This pattern appears to be characterized by consistently above average aggression across multiple settings, intense patterns of aggressive behavior, emotional lability, and a greater responsiveness to the introduction of psychological stresses into the environment. Results suggest early childhood may be an important period for <u>preventative and remedial intervention</u>. </p>		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project:

Carolyn Zahn-Waxler	Research Psychologist	LDP NIMH
Ronald Iannotti	Research Psychologist	LDP NIMH

Project Description:

Treatment programs for problem aggressive children have often not met with success, perhaps because they tend to begin too late. Recent reviews of the literature on children's aggression suggest that aggressive syndromes may originate in early childhood. However, children are seldom identified for treatment until after they enter school, often not until adolescence. Early intervention programs have obvious potential. To make this alternative successful, early aggressive syndromes and the factors that contribute to their development need to be better understood.

The present research program focuses on the nature and etiology of problem aggression in young children. A programmatic series of studies is ongoing that examines (a) the characteristics of early aggression, (b) its stability during development, and (c) its situational, environmental, and dispositional determinants and correlates.

In a first study the characteristics of children's aggression in the home was assessed on the basis of mothers' narrative reports of aggressive incidents. The children were followed between 1 and 2 1/2 years of age. The central finding was that stylistic characteristics of aggression, in particular the disposition to engage in intense displays of aggression, tended to be stable over time.

In a second study, specific hypotheses regarding situational, familial, and dispositional determinants of aggression were tested in a series of procedures administered to children at age 2 and again at 5. (a) In one laboratory procedure, play behavior between peers was recorded as the affective background environment was altered. Either friendly or angry interactions between two adults were introduced. (b) Children's aggression in peer interaction was analyzed in relation to mothers' reported rearing practices vis-a-vis aggression and (c) in relation to psychiatric and psychometric information on both mothers and children. Findings suggest the existence of an early aggressive pattern in young boys. This pattern appears to be characterized by consistently above average aggression across multiple settings, intense aggressive behavior, and emotional lability. These children are more likely than others to show altered behavior following the introduction of changes in the affective background environment. Aggressive boys were more likely than others to increase their aggressiveness following exposure to a background of angry interactions. Aggressive patterns are not as well-delineated for girls. Further analyses are ongoing.

Study three, now in a preliminary phase, focuses on two-to-three-year-old problem-aggressive children. Here the concern is again with the nature of early aggression and factors associated with its development. Autonomic response patterns in relation to aggressive behavior will also be studied.

The results of our studies to date suggest that well-delineated and stable syndromes are evident among some boys as early two years of age. Early childhood may be an optimum period for preventive intervention. The typical failure of treatment programs introduced in middle childhood or adolescence does not appear remarkable in light of these results. By the time treatment is begun, aggressive syndromes are likely to have been central elements of children's functioning for many years.

Significance to Biomedical Research:

This research program should provide partial answers to the following clinically significant issues: (1) What dispositional and experiential factors are associated with the early development of aggression? (2) What is the prognosis for change among young aggressive children? Risk profiles for young children and early intervention and prevention strategies are long-term goals.

Proposed Course :

Data collection for the first study and the first phase of the second study is complete. Manuscripts are currently in preparation, others under review for scientific journals, and reports have been presented at professional conferences. Follow-up testing of 5-year-olds in the second study will be finished this year. The third study is in the planning stages; testing will begin this year.

Publications:

Cummings, E. M., Zahn-Waxler, C., Radke-Yarrow, M.: Developmental changes in children's reactions to anger in the home. J. of Child Psychol. Psychiatry 24: 1-12, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02147-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects and determinants of parental methods for controlling children's behavior		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
Leon Kuczynski	Visiting Associate	LDP NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .20	PROFESSIONAL: .05	OTHER: .15
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This study investigates the determinants, content, and effects of parental disciplinary interventions with children. Parental control is an important focus of research on the environmental transmission of normal and dysfunctional behavior patterns. It was proposed that parents' choice of strategy may in part be determined by the kinds of goals they have when intervening to control a child's behavior. It was hypothesized that children's transgressions differ in the extent to which they arouse in parents goals for producing short-term, immediate changes of long-term enduring changes in children's behavior and that parents take these objectives into account when choosing strategies. </p> <p> A non-clinical sample of 64 middle-class mothers and their four-year-old children was studied in a laboratory experiment in which mothers were asked to have the child perform a monotonous task. Mothers' expectations of the long-term or short-term compliance required of their children was experimentally varied and the effects of these perceptions on a subsequent parent-child interaction were assessed. The results indicated that for long-term compliance goals, mothers tended to use more reasoning, character attributions and nurturance than for short-term goals. The same amount of power assertion was used in both conditions; however, boys received more power assertive techniques than girls. Strategies used in the long-term condition were also more effective in promoting both immediate and long-term compliance in children. Implications of this research for models of normal and disturbed parental functioning are discussed. </p>		

Names, Titles, Laboratory and Institute Affiliations of Other ProfessionalPersonnel Engaged on the Project:

None

Project Description:

This study investigates the determinants, content, and effects of parental disciplinary interventions with young children. Parental control is an important focus of research on the environmental transmission of normal and dysfunctional behavior patterns. Oppositional child behavior is one of the most frequent behaviors targeted for change in clinical interventions with families with problem children. Clinical interventions often directly focus on changing parental disciplinary strategies that may promote aggressive and noncompliant behavior in children. Current conceptualizations of parental disciplinary practices are inadequate in that they characterize parents in terms of a limited number of strategies (e.g. power assertive rewards and punishments vs. reasoning) that are assumed to be consistently used in all situations. Clinical interventions share a similar model in that parents are trained to use a limited number of strategies without empirically based guidelines concerning the situations in which specific strategies are appropriate. Such an approach is problematic because studies of nonclinical populations suggest that parents' choices of disciplinary strategies are strongly determined by situational variables. It is possible that the success of a strategy depends on when and how it is used; the success or failure of children's socialization experiences may depend on parents' abilities to adapt strategies appropriately to different situations.

In the present study, it was proposed that parents' choice of strategy may in part be determined by the kinds of goals they have when intervening to control a child's behavior. It was hypothesized that children's transgressions differ in the extent to which they arouse in parents goals for producing short-term immediate changes or long-term enduring changes in children's behavior and that parents take these objectives into account when choosing strategies.

A non-clinical sample of 64 middle-class mothers and their four-year-old children was studied in a laboratory experiment in which mothers were asked to have the child perform a monotonous task of sorting objects. Mothers' expectations were experimentally varied. A mother was cued either to expect that short-term compliance in her presence was needed or that long-term compliance that would endure in her absence was needed. The techniques used in these two circumstances and the effects of different strategies on children's behavior were assessed during a subsequent interaction.

The results indicate that, in the long-term condition, mothers were more likely to use a pattern of strategies including reasoning and explanations for parental directives, nurturance and feedback that attributed children's behavior to positive character traits (e.g. helpfulness, friendliness, intelligence, etc. than in the short-term condition. The same number of power assertive techniques (e.g. commands, aversive consequences, bargaining) was used in both conditions, but more power assertion was used with boys than with girls. Overall, the patterns of strategies used in the long-term condition were more effective than those of the short-term condition. Children in the long-term condition behaved in a more affectively

positive manner and were more compliant both immediately and in the mothers' absence. Children in the short-term condition were less compliant even in tests of short-term compliance and boys were more verbally oppositional.

The results indicate that parents use strategies for controlling children's behaviors that are qualitatively different in form and effect depending on the nature of their socialization goals in specific situations. An implication is that adaptive and maladaptive patterns of control may, in part, depend on the discriminations parents make between situations and their choice of strategies for achieving different goals. This hypothesis is currently being followed up in a study with child abusing and non-abusing parents (# MH 02158) and in a study of families with parents with and without diagnosed affective disorders (# MH 02144).

Significance to Biomedical Research and the Program of the Institute

The goal of this research is to understand the role of parental disciplinary practices in the development of competent patterns of children's behavior and their role in the etiology and treatment of childhood behavior disorders. By establishing empirical criteria for defining adaptive patterns of parental behavior, such studies may also open new lines of research that will contribute to the development of prevention strategies, i.e. providing parents with effective patterns of discipline and the development of clinical intervention strategies to assist parents in dealing with children with behavior problems.

Proposed Course

The findings of this study have been submitted for publication to the Journal of Developmental Psychology. The project has been completed.

Publications

Kuczynski, L.: Intensity and orientation of reasoning: Motivational determinants of children's compliance to verbal rationales. J. Exp. Child Psychol. 34: 357-370, 1982.

Kuczynski, L.: Reasoning prohibitions and motivations for compliance. Dev. Psychol. 19: 126-134, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02148-04 LDP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Physiologic Jaundice as a Predictor of Behavioral Function in Preterm Infants

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Sarah L. Friedman, Research Psychologist, LDP, NIMH

COOPERATING UNITS (if any)

Food and Drug Administration

Washington Hospital Center

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

.05

PROFESSIONAL:

.05

OTHER:

.00

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither☒ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Physiologic jaundice occurs when bilirubin, a breakdown product of the red blood cells, is not detoxified at the appropriate rate. Bilirubin levels in preterm infants are often elevated. This can result in damage to various parts of the brain and can consequently affect a range of behaviors. In this study we wished to determine if the subtle impairment in sensory, neurological, and affective function of low risk preterm infants is associated with physiologic jaundice. Bilirubin to protein levels in the blood of 45 low risk black preterm infants were correlated with four measures of tactile, auditory, and visual information processing, with performance on the Parmelee Neurological Examination, with observed activity level, with observed state and state-change, with length of observed crying and fussing, with two measures of ease of soothing and with judgments of acoustic features of the infants' cries. Of the 24 behavioral measures, 6 correlated significantly with the bilirubin to protein levels. Bilirubin to protein levels were associated with more time spent asleep and with a slower responsiveness to a visual stimulus. The pattern of the results did not change when either the effects of length of phototherapy (a treatment for jaundice) or the effect of other medical risks were statistically removed. The findings raise questions about the specific mechanisms through which bilirubin affects later visual responsiveness and state regulation in preterms (e.g. destruction of brain cells, degradation of myelin sheath, changes in neurotransmitters?). Likewise, the findings raise questions about the timing of phototherapy (preventive vs. responsive phototherapy) in the treatment of young preterm infants.

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Morris Waxler	Psychologist	FDA	DHHS
Milton W. Werthmann, Jr.	Director of Pediatrics	Washington Hospital Center	

Project Description:

I. Rationale and objectives

Physiologic jaundice occurs when bilirubin, a breakdown product of the red blood cells is not detoxified at the appropriate rate. Bilirubin accumulates in all neonates but for most infants this is not a serious problem because they produce a liver enzyme, glucuronyl transferase, which detoxifies the bilirubin. However, when an infant is born prematurely, its liver is not sufficiently mature to produce adequate quantities of the liver enzyme to detoxify the bilirubin present in the blood stream. Consequently, the bilirubin crosses the blood brain barrier and negatively and irreversibly affects various brain areas. It is known that bilirubin diffuses in the retina and the superior colliculi and that it damages motor pathways in the basal ganglia, brain stem and cerebellum. Such damage may lead to impaired visual and neurological function in affected infants. It is also known that high levels of bilirubin may produce serious cognitive, affective, sensory and motor deficits. For example, infants who survive severe neonatal jaundice often are afflicted by Kernicterus and are mentally retarded. Those with less jaundice are learning disabled. Consequently, it is reasonable to assume that still lesser levels of bilirubin may have more subtle but significant effects in different areas of functioning. In this study we wished to determine if the subtle impairment in the sensory, neurological and affective function of low risk preterm infants is associated with physiologic jaundice.

II. Methods employed

Subjects were 45 (24 male; 21 female) black, low medical risk (Hobel score \bar{X} = 25.0; S.D. = 18.0) preterm infants for whom measures of perinatal history were available. It is important to note that the behavioral function of the infants was measured many days after their treatment for jaundice was terminated (\bar{X} = 38.7 days; S.D. = 14.04). The physiologic jaundice of the infants was represented by the ratio of bilirubin to protein levels in the infants' blood stream. Two infants had no jaundice; thirty infants had a bilirubin/protein ratio smaller than 2.5; nine infants had a ratio between 2.5 and 2.9; four infants had a ratio equal to or greater than 3.0.

Sensory measures were used to evaluate the tactile, auditory and visual information processing by the infants. Quickness of response (latency), amount of responsiveness, amount of response decrement and duration of exposure to sensory stimulation were measured, using an habituation paradigm. Testing was

conducted independently in each of the three sensory modalities. Neurological function was evaluated using the Parmelee Neurological Exam and by recorded observations of state, state-change, and activity during a controlled observation procedure (\bar{X} = 901.7 seconds, S.D. = 388.5 seconds) in which the infant was looked at for periods of 5 seconds and behaviors were recorded live in the following 10-second periods. Affective function was evaluated by the percent of fussing, crying, ease of soothing during the observation period and by a blind experimenter's judgements of the acoustic qualities of the infants' cries.

III. Major findings

The results showed a relationship between the bilirubin to protein ratio and the following behavioral measures (1) quickness of response (latency) to the presentation of the visual stimulus (R = .40; P < .01); (2) duration of exposure to the visual stimulus (R = .40; P < .01); (3) the proportion of state transition during an observation period (R = -.33; P < .05); (4) the percent of time spent in active sleep during the observation period (R = .30; P < .05); (6) the percent of time spent in quiet wakefulness during the observation period (R = -.29; P < .05). Thus, physiological jaundice at the neonatal period was predictive of a depressed visual responsiveness, greater sleepiness, and reduced state change many days later.

IV. Significance to biomedical research and the program of the institute:

The findings lead to questions about the specific mechanisms through which bilirubin affects later visual responsiveness and state regulation in preterms (e.g. destruction of brain cells, degradation of myelin sheath, changes in neurotransmitters?). Likewise, our findings raise questions about the timing of phototherapy (preventive phototherapy versus responsive phototherapy) in the treatment of young preterm infants.

V. Proposed course:

To use the findings as presented in two conferences as a basis for a scientific paper. The paper will be submitted to a pediatric journal. Subsequently, the project will be terminated.

VI. Publications

Friedman, S. L. and Jacobs, B. S.: Wakefulness and visual responsiveness of low medical risk preterms. Early Child Dev. Care, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02150-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adjustment to Stress in Early Adolescence: Environmental & Organismic Factors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Editha D. Nottelmann Senior Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) Montgomery County Public Schools		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .80	PROFESSIONAL: .30	OTHER: .50
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This study examines the relationship between the occurrence of major transitions in the lives of children and their <u>psychological adjustment</u> . Children are studied in <u>transition</u> from elementary school to middle or junior high school and from <u>childhood</u> to <u>early adolescence</u> . Their adjustment in school before transition and their <u>ability</u> to withstand the <u>stress</u> of the changes they encounter during transition are examined in children's self-reports and reports from their teachers and peers. Analyses focus on (a) the range of psychological functioning in a normal volunteer sample of 445 children, (b) the incidence of <u>vulnerability</u> in terms of low self-esteem, poor self-image, isolation, and loneliness--factors implicated in the <u>affective disorders</u> leading to depression and suicide and in <u>acting out</u> behaviors that stabilize in mid-adolescence and peak in the 15- to 25-year age bracket.		

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel Engaged on the Project:

C. Jean Welsh

Psychologist

LDP

NIMH

Project Description:

This study examines the relationship between the occurrence of major transitions in the lives of children and their psychological adjustment. The transition from primary to intermediate school has been selected for study because it represents significant imposed change for a large number of children in our society; not only the change in schools, but also change from child to adolescent status. Moreover, although the transition does not coincide with the onset of puberty for every child, physical maturity is an issue that is imposed on all children as they join a more mature student body. The convergence of these changes makes early adolescence a period in which there is a likelihood of stress and vulnerability. Adjustment and adaptation to change are examined across school settings, from the children's own perspective and the perspective of their teachers and peers.

In a volunteer group of 445 children from a local school system, we are examining the range of psychological adjustment. We are focusing on self-esteem, peer-group standing, and level of performance in school, as well as on children's feelings of competence in the domains important to their age group. Measurements are made prior to transition, early in the period of transition, and again later, after they have had an opportunity to adapt to their new environment.

While transition itself was found to have no adverse effects on children entering middle or junior high school, "off-time" physical maturation appears to play an important role in children's adjustment. Girls who were relatively more mature (compared with most girls in their peer group) and boys who were relatively less mature (compared with most boys in their peer group) perceived themselves as less competent in interpersonal and athletic situations. These "off-time" children also reported relatively low self-esteem. Teachers rated these children as different from their peers only in physical competence (sports).

"Short-for-age" children also appear vulnerable. In a comparison of relatively short and tall stature children, short stature children reported lower self-esteem and academic competence than tall stature children. The children's assessments of their academic competence were supported by their teachers, who gave short stature children much lower ratings than tall stature children.

Children's physical competence ratings across the one-year transition period were found to be most stable; self-esteem, least stable. In keeping with the estimate that 10 to 20 percent of the adolescent population experiences some psychopathology, we are finding that about 15 to 20 percent of the children report low self-esteem and marginal peer relationships. Children's responses on a projective questionnaire indicate that approximately 18% of the children, more boys than girls, are likely to engage in fighting and bullying other children. Additional analyses will focus on the pre- and post-transition psychological profiles of these subgroups of children. Also, the possibility of follow-up study of these children is being explored.

Significance to Biomedical Research:

This research is yielding information on the normative distribution of psychological adjustment in a normal volunteer sample. It is documenting the incidence of marginal adjustment. It shows promise of identifying precursors of serious problems of adolescence; namely, depression and suicide; and of documenting the usefulness of self-report for early detection of adjustment problems and directing efforts of intervention and prevention of psychopathology.

Proposed Course:

Data evaluation is in progress. Preparation for publication and dissemination of the findings has begun and should be completed during the coming year.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02152-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Discipline and Parental Control in Families with Affective Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Leon Kuczynski Visiting Associate LDP NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .45	PROFESSIONAL: .25	OTHER: .20
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This study investigates the determinants, content and effects of <u>parental discipline and control practices</u> in families with normal and clinically depressed mothers. This study, the basic paradigm for which is described in Annual Report # MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with normal and affectively disturbed parents. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior, such as noncompliance, aggressiveness, and other antisocial behaviors. </p> <p> Assessments of parent and child behavior are based on detailed observations of parent-child interaction in a naturalistic setting. Specific aspects of parental control that are being investigated are the purpose or function of parental interventions, the quality and timing of mothers' strategies and their ability to resolve conflicts successfully after initial noncompliance in children. Children's self-control and emotional reactions to parental interventions are also assessed. A second focus is on the role of individual difference variables, social class of mother, and seriousness and current status of mothers' affective disorder that may mediate the impact of depression on parental functioning. </p>		

Names, Titles, Laboratory and Institute Affiliations of Other ProfessionalPersonnel Engaged on the Project:

Marian R. Yarrow

Chief

LDP

NIMH

Project Description:

This study investigates the determinants, contents and effects of parental discipline and control practices in families with normal and clinically depressed mothers. This study, the basic paradigm for which is described in Annual Report # MH 02144, is part of a series of investigations assessing the environmental transmission of competent and disordered patterns of child behavior in families with affectively disturbed parents. Impaired parental skills in managing children's behavior have repeatedly been implicated in the etiology of maladaptive patterns of child behavior such as noncompliance, aggressiveness and other antisocial behaviors. Skillful use of control and disciplinary strategies may promote personal and social competence in children.

The use of ineffective forms of discipline and control may be one consequence of parental depression. Several studies suggest that depressed mothers are less involved in the day to day control of their children and, when they do intervene, use punitive forms of discipline. However, this research has been global in nature and has not clarified the specific processes underlying the impairment in functioning. One purpose of this study is to provide a detailed behavioral assessment of the control practices of depressed and nondepressed mothers and to examine their effects on children's self-control, compliance and regulation of emotions. Specific aspects of parental control interventions that are being investigated are the purpose or function of parental interventions, the quality and timing of mothers' strategies and their ability to successfully influence their children's behavior when children initially resist complying. Parental choice of strategy in relation to specific categories of children's misbehaviors will also be assessed.

A second focus of the study is on the role of individual difference variables, social class of mother and the current status and seriousness of mothers' affective disorder, that may mediate the impact of depression on parental functioning. An important question is whether difficulty in the management of children's behavior is an enduring feature of depressed mothers or whether it consists of transient problems that are confined to the acute stages of the depressive episode. It is also possible that depression does not have the same impact across social class. Middle class women with social supports and resources not available to low social economic class women may be better able to cope with the added stress of depression and have their parental functioning less impaired by depression than low class mothers. An analysis of the data by social class and by diagnostic category and timing of the affective disorder is being made.

Significance to Biomedical Research

Children of depressed parents have been found to be at greater risk for psychopathology and behavioral disorders than children of normal parents. Early research suggests that aberrant disciplinary practices comprise one of a pattern of environmental factors to which such children are exposed. The present study investigates

the role of discipline in families with affective disorders both as a process that may predispose children to behavioral problems and also as a focus for new forms of prevention and treatment.

Proposed Course

Data for this project and for other projects in this series are being collected from observations of normal and clinically depressed mothers and their children in a series of half-day sessions in a laboratory setting designed to simulate a home environment. Data collection and analysis is underway. Instruments for coding the videotaped data are being developed.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02153-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Maternal Recall of Child's Early Experience		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Penelope K. Trickett Senior Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) NONE		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years: .45	PROFESSIONAL: .15	OTHER: .30
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Mothers' retrospective reports of certain <u>child behaviors</u> and <u>child-rearing techniques</u> and parallel information obtained at an earlier time when the behaviors were current are investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Although previous research has demonstrated that in general the correspondence between contemporaneous and retrospective data is low, there is still much to be learned about what determines when mothers' recollections are more or less accurate. (1) Are certain kinds of information recollected more accurately than others? (2) How does the mother's current view of the child color her memories? (3) Does depression in the mother affect her recollections? The subjects are mothers of 26-month-old children who are part of a study of etiology of behavior problems. Data collection and coding are completed and statistical analyses are underway.		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project:

Marian R. Yarrow
Carolyn Z. Waxler

Chief
Research Psychologist

LDP NIMH
LDP NIMH

Project Description:

Much of the available knowledge on childrearing techniques and psychopathology in early childhood has been derived from information obtained by interviewing mothers. Many of these interviews have been retrospective, asking the mother to recall certain characteristics of her child or her child-rearing techniques when her child was at a younger age. Other interviews concentrate on the present but still rely on retrospection to the extent that they require the mother to use memories of previous experiences in order to make generalizations about her own or her child's typical behavior. In this study, the relation between mothers' reconstructions of certain child behaviors and child-rearing techniques and parallel information obtained at an earlier time is investigated. Issues of maternal recall of children's early characteristics are of special relevance in etiological questions of psychopathology. Previous research (Yarrow, Campbell, & Burton, 1970) has demonstrated that the correspondence between mothers' recollections and parallel information obtained at an earlier time is often low and that systematic biases in retrospection can occur. However, there is still much to be learned about the factors that determine when mothers' recollections and generalizations are apt to be more or less accurate. For example, are certain kinds of information recollected more accurately than others? How does the mother's current relationship to the child color her memories? How does depression in the mother affect her recollections?

Mothers in the current research are part of a clinical study of etiological factors in the development of behavior problems of children. The baseline data are observational records by mothers who have been trained as observers and have been providing, on a longitudinal basis (10 to 24 months) extensive data on their children, e.g., children's responses to stressful and pleasurable life events, their aggressiveness and noncompliance, and their prosocial behavior. Mothers also reported on their discipline techniques. When each child was 26 months of age, the mother provided retrospective accounts of her child's and her own behavior parallel to that which she recorded when the child was 18 months old. The SADS interview and DSM-III criteria were used to determine psychiatric diagnosis for each mother. Each mother also filled out a standard child-rearing attitudes measure and a mood checklist.

The collection and coding of all data have been completed. Preliminary analyses have been done to validate the interview measure by determining the relationship between interview responses concerning child characteristics and maternal discipline and independent assessments of child characteristics, obtained when the child was 26 months of age in a laboratory setting (Z01 MH 02146). The latter analyses indicate that the mother's retrospective reports of her child's temperament, anger, and aggression at 18 months of age were significantly correlated with measures of aggression with peers in the laboratory at 26 months of age. (The more irritable his/her temperament and the more angry and aggressive especially toward parents at 18 months, the higher the aggression toward a playmate at 26 months.) Also, mothers who reported high use of admonishment, verbal prohibition or scolding, and physical restraint as discipline techniques at 18 months had

children who were more aggressive to peers in the laboratory at 26 months. These discipline techniques may cause high levels of aggression or be a reaction to the aggressiveness. The findings may also represent a cumulative interactive effect in which high levels of child aggression and strong parental discipline each become integral parts of a coercive cycle: Some parents and children may become "locked in" to aggressive interchanges from the earliest years of life.

To answer the methodological research questions noted above, analyses are in progress. First, the accuracy of mothers' retrospections of different types of maternal and child behavior (such as affectively-charged aggressive behavior and more neutral or positive child behavior) are being determined. Then, multiple regression analyses will be conducted to determine to what degree maternal psychiatric diagnosis, mood, enjoyment of the parental role, and child-rearing attitude predict accuracy of retrospection for these different areas of child and maternal functioning.

Significance to Biomedical Research

This study will result in a delineation of both the types of information that are more or less accurately remembered by mothers and the characteristic of the mother's personality, attitudes and values which affect retrospection. Such a delineation will aid the development of subsequent biomedical and psychiatric research methods which rely on maternal retrospection as the method of choice for obtaining necessary information about children's past histories.

Proposed Course

Statistical analyses of the data are underway, a manuscript is in preparation, and the project should be completed within one year.

Publications:

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 02154-04 LDP

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Stability and change in behavior problems of clinically referred children

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jerome H. Blue

Staff Fellow

LDP

NIMH

COOPERATING UNITS (if any)

University of Vermont School of Medicine, Burlington, Vermont

California State University, San Luis Obispo, California

LAB/BRANCH

Laboratory of Developmental Psychology

SECTION

INSTITUTE AND LOCATION

National Institute of Mental Health, Bethesda, Maryland

TOTAL Staff Years

.40

PROFESSIONAL:

.30

OTHER:

.10

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This study assessed stability and lability of clinically relevant problem behaviors of children. The sample ($n = 468$) consisted of both boy and girl outpatients of a community child guidance clinic. Child Behavior Checklists of 6- to 11- and 12- to 16-year-olds were filled out by parents at intake and again 6 months and 18 months thereafter. Using a longitudinal analysis, the following derived from Achenbach's Child Behavior Checklist were examined: individual behavior problems, behavioral syndromes, and groupings of externalizing and internalizing behavior problems. Analyses showed that stability and lability of problem behaviors varied by age and sex. As a group, children showed decreases in the intensity of behavior problems, although maintaining stable rank ordering relative to peers.

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Thomas M. Achenbach	Research Psychologist	University of Vermont
David J. Cain	Psychologist	California State University

Project Description:

Efforts addressing the issue of stability and change in behavior problems of clinically referred children have provided conflicting results. It is possible, therefore, that unnecessary intervention for transitory behavior problems and lack of effective treatment for stable problems may occur in clinical settings. Therefore, in order to help guide treatment decisions toward the most critical behavior problems, data of children referred for mental health services were analyzed. The Child Behavior Checklist, a standardized inventory for assessing change in behavior problems was used.

Methods Employed:

The participants were 81% white and 19% black from an outpatient community child guidance clinic. They had an average Hollingshead socioeconomic score of 4.6 for breadwinners' occupation. Because stability and change in specific items is likely to vary with the sex and developmental level of the children, as well as the interval assessed, separate analyses were performed at 6- and 18-month intervals for each sex at ages 6 to 11 and 12 to 16. Furthermore, because children as a group may show increases in the intensity of a particular problem behavior while maintaining stable rank orders relative to their peers, we computed changes in mean scores as well as correlation coefficients. Analyses were performed on: (1) 118 individual behavior problems, (2) behavioral syndromes, robust factors showing groupings that take into account the sex and age differences in the patterning of behavior problems, and (3) externalizing and internalizing behavior problems which are second order factor analyses of groupings of aggressive and undercontrolled behaviors (externalizing) and groupings of fearful and overcontrolled behaviors (internalizing).

Major Findings:

The follow-up assessments used in this study revealed that there are behaviors that are resistant to change for both sexes aged 6 to 11 and 12 to 16. Also, there were behavior problems that were stable for both sexes, but not for both age groups. Younger boys had more stable behavioral syndromes than older boys, but there was no difference in the number of stable syndromes between younger and older girls.

The assessment of externalizing and internalizing problems showed that at younger ages girls had more stable internalizing behavior problems than boys; at older ages girls had more stable externalizing problems than boys.

Significance to Biomedical Research

The findings show that the question of stability and change in clinically relevant behaviors cannot be answered in an all-or-none fashion. Global assessments of problem behaviors may overlook many serious behavior problems that are resistant to change. However, assessments of individual behavior problems can show the particular behaviors that are most serious and resistant to treatment. The research provides guidelines for identifying behavior problems resistant to change.

Proposed Course

The project is completed. A manuscript is being submitted for publication.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02155-04 LDP
PERIOD COVERED <u>October 1, 1982 through September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Children of Depressed and Normal Parents</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Carolyn Zahn-Waxler</u> <u>Research Psychologist</u> <u>LDP NIMH</u>		
COOPERATING UNITS (if any) <u>None</u>		
LAB/BRANCH <u>Laboratory of Developmental Psychology, NIMH</u>		
SECTION 		
INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</u>		
TOTAL Staff Years <u>1.65</u>	PROFESSIONAL: <u>.90</u>	OTHER: <u>.75</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Social-emotional competence, coping, and vulnerability are assessed in a longitudinal study of young children with an affectively ill parent. The sample consists of 48 predominantly middle-class families. Children of mothers with unipolar depression (current major, past major, or past minor) and normal mothers are seen at two and at five years of age. They are observed in a laboratory setting in interaction with their mothers, adult strangers, and familiar playmates. The affective environment is varied experimentally to assess children's social-emotional responses. An earlier investigation using similar procedures to study two-year-old children with a bipolar parent indicated significant problems in the quality of interpersonal relations, ability to empathize with playmates, and in resolution of hostile impulses. Analysis are underway to determine (a) whether similar social-emotional problems characterize children with parents with unipolar depression and (b) whether the severity and chronicity of parental illness influence the child's functioning. Comparisons are made of the children's behavior at two and five years in order to explore processes associated with continuities and discontinuities in social-emotional patterns of coping in children with an affectively ill parent.</u>		

Names, titles, laboratory and institute affiliation of other professional personnel engaged on the project:

Marian Radke-Yarrow	Chief	LDP NIMH
E. Mark Cummings	Staff Fellow	LDP NIMH
Dale Hay	Guest Worker	LDP NIMH
Ronald Iannotti	Research Psychologist	LDP NIMH

Project Description:

Although offspring studies have demonstrated the familial aggregation of affective illness, not all depressed parents have children with emotional problems nor do depressed offspring inevitably have an affectively ill parent. The moderate degree of correspondence of emotional problems in parents and their children in offspring studies indicates both the need to identify those biological and environmental processes that contribute to patterns of concordance and also those processes that contribute to exceptions in intergenerational transmission of affective disorders. The offspring studies also indicate the need to examine the early evolution of symptoms in children, in order to identify possible precursors of diagnosable affective illness.

Core features of adult depression include disturbances in regulation of emotion, problems in social relations, and feelings of helplessness, all of which may combine to produce lowered levels of social-emotional competence in a parent. Chronic exposure to such symptoms might be expected to influence children's own social-emotional functioning and to result, sometimes, in parallel problems to those of the parent. The first purpose of this research is to identify, early in development, adaptive and maladaptive patterns of social-emotional functioning in children with a depressed parent. The second purpose is to explore, through longitudinal assessments, the processes by which (a) early problems do or do not later culminate in diagnosable disturbances and (b) early social-emotional competence does or does not predict later adaptive functioning. To examine these issues, children's regulation of affect, social competence and vulnerability, psychiatric status, and capacities for negotiation and conflict resolution are explored.

Methods Employed and Major Findings:

Young children of normal mothers and mothers with diagnoses of unipolar depression (current major depression, past major depression or past minor depression) are compared. DSM-III criteria were used to diagnose depression. Measures of moods, personality styles, and level of social functioning are also obtained on the mother. The children were first observed as two-year-olds, in interaction with their mothers, adult strangers, and familiar playmates in laboratory settings. Their adaptive and maladaptive patterns of aggression, empathy, affiliative behavior and emotion regulation were examined under a range of experimental conditions designed to create pleasure, challenge, conflict, and distress. A small sample of two-year-old children with a bipolar parent exposed to these procedures had shown multiple deficits (Z01 MH 02135-05 LDP). These children were likely to have difficulties in sharing, in maintaining friendly relations with playmates, and in modulating emotions of anger and sadness. They were also insecure in their relationships with their mothers and generally showed pre-occupation with the problems of adults. These latter deficits may interfere with children's ability to establish stable peer relations early in life. Data analysis

is underway to determine (a) whether similar social-emotional problems characterize two-year-old children of parents with unipolar depression, and (b) whether the severity and recency of parental illness influence the child's functioning. Preliminary analyses indicate that many of the social-emotional problems that characterize children of manic depressives do not similarly typify children of a unipolar depressed parent. There is an exception to this generalization: Children with a depressed parent (regardless of type of depression) are consistently less likely to show physical aggression toward playmates than are children with normal mothers.

Comparable research procedures are used to study these children again at age five. The Beck Depression Inventory and a mood scale is administered to both mothers and fathers. Mothers complete the Achenbach symptom check list on the children and clinicians make psychiatric assessments as well. Structured tests are used to measure, in hypothetical situations, dimensions of children's functioning that have parallels in the observational procedures (i.e., coping strategies, moods, aggression, and empathy). Cognitive performance is also examined.

Data collection on the 5-year-olds has begun. Preliminary observations indicate that 5-year-olds employ a great range of strategies for dealing with challenge and conflict: Some children already can be seen to use humor to make a threatening situation less stressful or to use the relationship with a friend as a source of support. Other children show patterns of avoidance, denial, and projection. Deterioration of symbolic play in the presence of external stressors for some children is noted. These distinctively different coping styles will be examined in relation to parental characteristics.

Significance to Biomedical Research and the Program of the Institute

Careful description and analysis of how children regulate their emotions, cope with stressful life events, negotiate interpersonal problems and develop close relationships may help to understand the etiology of affective problems in children. Knowledge of processes contributing to these abilities should help to explain both why mental illness persistently characterizes successive generations in some families and also how some children from disturbed families learn to master situations of conflict and distress and hence to begin to break the cycle of illness.

Proposed course:

The follow-up of five-year-olds will be carried out over a six-month period. Analyses and reports follow.

Publications:

Zahn-Waxler, C., Friedman, S., and Cummings, E. M.: Children's emotions and behaviors in response to infants' cries. Child Dev., in press.

Zahn-Waxler, C., Radke-Yarrow, M., and King, R.: Early altruism and guilt. Acad. Psychol. Bull., in press.

Zahn-Waxler, C., Cummings, E. M., and Cooperman, G.: Emotional development in childhood. Annals of Child Dev., in press

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02156-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Emotional-Social Development of Children Reared by Normal and Depressed Mothers		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Marian Radke-Yarrow Chief LDP NIMH		
COOPERATING UNITS <i>(if any)</i> None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years 3.05	PROFESSIONAL: 1.65	OTHER: 1.40
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> A program of coordinated studies is designed to investigate child development in families with and without <u>affective disorders</u> . The program has four interrelated components. One is the delineation of attributes of pathological and normal rearing environments. <u>Maternal behavior and parental child interactional patterns</u> are investigated with regard to <u>affective qualities</u> (in content, form, and function), <u>cognitive properties</u> , and <u>control or regulatory aspects</u> (also in content, form, and function). The second component is the assessment of children in terms of their cognitive, affective, and interpersonal skills and dysfunctions. The third objective is to treat the study as an <u>offspring study</u> , comparing diagnoses of children with diagnoses of parents. The fourth and major component is the investigation of processes of <u>transmission of adaptive and maladaptive behavior patterns</u> in children. This entails study of the multiple pathways of influence and the varied repertoires of coping in rearing and being reared. By using direct observation over time, this study brings to "offspring" research some of the data necessary to understand the kinds of learning experiences out of which behavior develops, in interaction with predispositions in the child.		

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Leon Kuczynski	Visiting Associate	LDP	NIMH
Leon Cytryn	Research Psychiatrist	LDP	NIMH
Donald McKnew	Research Psychiatrist	LDP	NIMH
Sarah Friedman	Research Psychologist	LDP	NIMH
Barbara Hollenbeck	Research Social Science Analyst	LDP	NIMH
Judy Stilwell	Social Science Analyst	LDP	NIMH
Mark Cummings	Research Psychologist	LDP	NIMH
Ronald Iannotti	Research Psychologist	LDP	NIMH
Ruth Wylie	Guest Worker	LDP	NIMH

Project Description

A program of coordinated studies is designed to investigate child development in families with and without affective disorders. The program has four interrelated components. One is the delineation of attributes of pathological and normal rearing environments. Maternal behavior and parental child interactional patterns are investigated with regard to affective qualities (in content, form, and function), cognitive properties, and control or regulatory aspects (also in content, form, and function). The second component is the assessment of children in terms of their cognitive, affective, and interpersonal skills and dysfunctions. The third objective is to treat the study as an offspring study, comparing diagnoses of children with diagnoses of parents. The fourth and major component is the investigation of processes of transmission of adaptive and maladaptive behavior patterns in children. This entails study of the multiple pathways of influence and the varied repertoires of coping in rearing and being reared. By using direct observation over time, this study brings to "offspring" research some of the data necessary to understand the kinds of learning experiences out of which behavior develops, in interaction with predispositions in the child.

Parents diagnosed as depressed (bipolar, major unipolar, minor unipolar and intermittent) and normal parents and two of their children (ages 15 months to 2 years and an older sibling 5 to 8 years) are the research participants. Parents are diagnosed using Schedule for Affective Disorders and Schizophrenia, life-time version (SADS-L) and the associated Research Diagnostic Criteria (RDC). The mother's rearing behavior, her actions and her interactions with her children, and the children's functioning are examined directly.

Mothers and children are observed with each other over a series of half-days, providing naturalistic and experimental data. They are also interviewed and tested. After a year and a half, assessments are again made. Children are seen also with peers. Psychiatric assessments are obtained on each child, psychiatrists being blind as to mother's diagnosis and to observed parent and child behavior. (The details of the research paradigm are described in Z01 MH 02144.)

A number of analysis objectives within the broader study have been developed. Thus far, most of them concern parental behavioral variables. These several analyses address the following issues: (a) One possible route to dysphoria in the young child is through maternal techniques that lower the child's self-esteem. Maternal verbal attributions to the child are analyzed for contents and frequency. Very preliminary data indicate relatively infrequent explicit negative or positive attributions by normal mothers but a very high level of implicit messages regarding the child's adequacy or inadequacy. (b) Analyses are in progress regarding the mother's handling of anticipated and experienced emotional distress in the child, by examining affective communications, cognitive components, and mother's manipulation of the situation. (c) In an earlier study of manic-depressive and normal mothers, disturbed attachment relationships were found with greater frequency among depressed than among normal mother-child pairs. Similar analyses are underway in the present study. Further, the quality of attachment (measured in the Ainsworth Strange Situation) is analyzed in relation to other indicators of attachment, the child's social relationships and affect regulation. (d) Mothers' expressions of affection and their encouragement or discouragement of affectionate expression in their children are analyzed with reference to mothers' diagnoses. The forms of affection and the functions of affection are examined. (e) Differences are found between bipolar mothers' and normal mothers' orientations toward their children and their rearing objectives, particularly on dimensions concerning affect. The affectively ill mothers are more likely to have negative feelings toward their children but less likely to express their feelings openly. They tend to be less likely to encourage openness to new experiences, and to be more highly protective. They tend to stress the child's control of his/her feelings. Although these are only group differences and a first level of analysis, they suggest very early shaping of the child's affective experiences and affective expression.

Significance to Biomedical Research

This program of studies will provide information relevant to an understanding of familial environmental factors that predispose young children to, or protect them from psychological disorders. It will attempt to identify attributes in children that contribute to these processes. The focus is on very early development, with special reference, therefore, to early detection, prevention, and treatment. This research is one of few systematic studies that investigate in a direct way the functioning of depressed parents and the effects on young children.

Proposed Course

This is a longitudinal study in which families are followed over a period of 3 years. The sample is now about 80 families. It will be increased in order to fill certain categories of diagnosis, age of child, and social class of family. Analyses not involved in the longitudinal aspects are now underway with reports being prepared for publication.

Publications

Cytryn, L., McKnew, D.H., Jr., Zahn-Waxler, C., Radke-Yarrow, M., Gaensbauer, T.J., Harmon, R.J., and Lamour, M.: Affective disturbances in the offspring of affectively ill parents - a developmental view. Am. J. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02157-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Evaluation of Infants on Chloride Deficient Diet		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> Howard A. Moss Guest Worker LDP NIMH </div>		
COOPERATING UNITS (if any) Pediatric Metabolism Branch, NIAMDD		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION 		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years: .15	PROFESSIONAL: .10	OTHER: .05
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>In 1979 it was reported that a group of infants who were receiving a <u>Soy formula</u> were not behaving and developing according to normative expectations and were exhibiting a "failure to thrive" type syndrome. Analysis of this infant formula revealed that it was deficient in chloride and that many of the infants fed this formula were suffering from <u>metabolic alkalosis</u> and chloride and potassium deficiencies. Changes to a properly balanced formula improved their condition. There was some question, however, whether or not any permanent damage was sustained. The mental and psycho-motor development of a small sample of these children was studied at 2 years and 3 1/2 years. There was some suggestion of a developmental deficit at the earlier assessment but by 3 1/2 years these children appeared to be functioning at their appropriate developmental level.</p>		
(479)		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project

Van S. Hubbard

Clinical Associate

PMB NIAMDD

Project Description:

In 1979 it was reported in an investigation conducted by the Center for Disease Control in Atlanta, Georgia, that a number of infants who were receiving a chloride deficient formula as their primary source of nourishment exhibited physical and behavioral abnormalities. These abnormalities consisted of weight loss, failure to grow, muscular weakness, delayed motor and speech development, chloride and potassium deficiencies, and a condition known as metabolic alkalosis in which the pH levels of the blood are excessively alkaline. To date, 141 documented cases of infants who were fed chloride deficient formulas have exhibited at least one episode of metabolic alkalosis. Analyses of these formulas have shown their chloride levels were about one-fifth of the amount recommended for infants by the American Academy of Pediatrics.

Once the formula was corrected there was remission of the metabolic abnormalities. The purpose of the research is to determine if there are any continuing complications or long-term sequelae associated with the past use of the chloride deficient infant formulas. This project is under the direction of Dr. Van Hubbard (NIH-NIAMDD) and consists of evaluations of the physical and psychological growth of these infants as well as evaluations of their biochemical, physiological, and neurological status, carried out at yearly intervals over several years. The Laboratory of Developmental Psychology is responsible for the psychological assessment of these children.

This study consisted of two assessments of children who were fed these formulas and developed metabolic alkalosis. One assessment was at approximately 24 months of age and the second one at about 42 months of age. Both assessments have been completed. In the first assessment the Bayley Scales of Infant Development were administered and the second assessment the McCarthy Scale of Intelligence was used. The McCarthy scales provide scores on memory, attention, language abilities, and motor skills--functions about which concern has been expressed. Siblings were tested on the follow-up assessment and serve as a control group. The two testings of the patient group provide the opportunity to determine if changes occurred over time in the level of functioning of these children.

In the first assessment there was some indication that the amount of time that a child was exclusively on a chloride deficient diet was associated with lowered mental abilities, however, for the second testing these children performed within the normal range and did not differ from the performance levels obtained by their healthy siblings.

Significance to Biomedical Research:

This research has relevance for determining if a chloride deficient diet during an early and critical stage of development has any long term and progressive effects on neuropsychological functioning. This research is important not only for providing data on the interaction between diet and mental status, but for monitoring possible deleterious effects and for determining if intervention and compensatory training are required.

Proposed Course:

Data collection has been completed. The results of this research will be included in a publication that is in preparation and the current protocol will be terminated.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02158-04 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Impact of the Environment on the Development of the Abused Child		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Penelope K. Trickett Senior Staff Fellow LDP/NIMH		
COOPERATING UNITS (if any) Agencies and Institutions in the Washington Metropolitan area serving abusing families		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years: 1.85	PROFESSIONAL: 1.10	OTHER: .75
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This study focuses on the <u>psychological development</u> of <u>physically abused children</u> and the relationship between this development and the child-rearing environment of the home. While clinical evidence shows that abused children are at risk for a wide range of psychological problems, few controlled empirical studies exist and there is no research relating aspects of the <u>enduring environment</u> of the abused child to the child's development. This study uses a <u>multi-method</u> approach to obtain information. The child's development is assessed in relation to <u>cognitive</u> and <u>physical maturity</u>, <u>affective behavior</u>, <u>interpersonal problem solving skills</u>, and <u>behavior problems</u>. The child-rearing environment is assessed in terms of psychosocial environment of the home, family social network and social supports, parental frustration tolerance, child-rearing attitudes and practices and parental mood. <u>Psychopathology</u> in the <u>parents</u> is also assessed. Subjects are 4- to 10-year-old abused and control children and their families. Preliminary analyses indicate greater behavior problems in the abused children, more punitive and authoritarian child-rearing attitudes and practices in the abusive parents, and more depressed mood and smaller social networks for abusive mothers than for controls. </p>		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project

Elizabeth J. Susman

Senior Staff Fellow

LDP/NIMH

Project Description

This study focuses on the psychological and behavioral development of physically abused children and the relationship between this development and aspects of the rearing environment of the child including the presence of psychopathology in the parents. It is being conducted within a framework which includes characteristics of the abused child, the abusive parent, and the family environment and is based on the premise that both the causes of and the impact of child abuse can be understood only by considering physical abuse within the context of the enduring parent-child relationship or child-rearing environment.

One purpose of the study is to characterize the psychological development of abused children. While there is wide agreement that the childhood victims of physical abuse are at risk for later behavioral maladjustment, few controlled empirical studies bearing on this issue exist.

A second purpose is to investigate the childrearing environment of abusive families with particular attention to those processes which may lead to abusive incidents. There is evidence that frequently the immediate antecedents of parental abuse involve parental discipline attempts or other efforts at controlling the child. However, the exact nature of this relationship between abuse and discipline is unclear. Two competing hypotheses are (1) that abusive parents may believe that harsh punishment is a necessary technique if one is to rear a child adequately and (2) that abusive parents, while not valuing physical punishment any more than other parents, tend toward out-of-control anger episodes set off by child misbehavior. What contribution parental psychopathology may play in triggering abusive episodes is unknown and is also a focus of study.

A third purpose is to investigate relationships between the child-rearing variables and the development of the abused child. As suggested earlier, the child's development is likely to be affected not just by the sporadic episodes of physical abuse, per se, but by the more enduring child-rearing environment of the home.

Subjects are physically abused children ranging in age from 4- to 10-years and their parents. They are recruited from protective service agencies in the Washington Metropolitan area. Criteria for inclusion in the sample are being either a single- or two-parent family in which at least one of the parents is the abuser. A control group of non-abusing families is recruited from community agencies. These families are matched to the abusing families on age, race, and sex of the child and educational and occupational status of the parents. The total sample is 60 families.

This study uses a multi-method approach to obtain information about the social and emotional development of the child and of the child-rearing environment of the home. Standardized measures are used to assess the child's development in the areas of cognitive (Peabody Picture Vocabulary Test) and physical (Bruininks-Oseretsky Scale of Motor Development) functioning, social problem solving skills (Preschool Interpersonal Problem Solving Skills and Open Middle Test), and

behavior problems (Achenbach Child Behavior Checklist). Affective coping style and predominant mode of relating to family members are assessed by observational methods.

To assess the child-rearing environment a combination of standardized measures and observational methods is used. The variables measured include family psychosocial environment (Moos Family Environment Scale and Social Network Analysis), parental frustration tolerance (Rothbart-Maccoby Role Playing Measure and observational methods), parental childrearing attitudes, values and practices (Block Child-Rearing Practices Q-Sort and parent record-keeping), parent-child interaction (observational methods), parental mood (Profile of Mood Scales), and parental psychiatric diagnosis (SADS).

Preliminary analyses indicate that, as measured by the Achenbach Child Behavior Checklist, the abused children have a significantly higher number of behavior problems than do the control children. This is true for both the Internalizing and Externalizing Subscales of this measure but is more pronounced for the Externalizing Subscale where 79% of the abused children score above the 98th percentile as compared with 21% of the control children. Also, on the social problem solving measures, the abused children are significantly more likely to suggest forceful solutions to interpersonal problems than are the control children.

These early analyses also indicate differences in the abusive and control families both in child-rearing attitudes and in their records of actual discipline practices. Both of these types of data show that abusive parents believe in and use more and harsher physical punishment than do control families (although an equally high majority of both groups report using physical punishment at times). Also, abusive families place less value on and less frequently use reasoning as a discipline technique. Other differences in child-rearing environment have also been found in these preliminary analyses. First, as measured by the Profile of Mood Scale, abusive mothers are more depressed than control mothers. This relationship does not hold for abusive and control fathers. Second, abusive mothers report a smaller social network than do control mothers. (Again, this is not so for fathers.)

Significance to Biomedical Research:

This study addresses two distinct etiological issues. One focus is on the causes of child abuse with particular emphasis on the role played by parental psychopathology and parental child-rearing attitudes and behavior. The second focus is on the effect of child abuse on the psychological development of the victims. Information on these two issues can aid greatly in the development of treatment programs for abusive families and preventive interventions.

Proposed Course:

Data collection is complete. The coding and analysis of the data are underway. One manuscript, "Heterogeneity in Children's Responses to Similar Abusive Environments" has been completed and submitted for publication. Work on other manuscripts has begun and the project should be completed within one to two years.

Publications:

Trickett, P.K.: The interaction of cognitive styles and classroom environment in determining first-graders' behavior. J. Appl. Develop. Psychol., in press.

Trickett, E. J., Trickett, P. K., Castro, J. J., and Schaffner, P. The independent school experience: Aspects of the normative environments of single sex and co-ed secondary schools. J. Educ. Psychol. 74: 374-381, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02159-03
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Information Processing and Adaptation to Research Hospitalization		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
Elizabeth J. Susman Senior Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) Office of the Director, Clinical Center		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years .65	PROFESSIONAL: .10	OTHER: .55
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This study focuses on the <u>cognitive functioning</u> of patients in hospital-based clinical research. Participation in medical research is stressful for most patients. Therefore, the <u>informed consent</u> procedure is executed in relatively stressful circumstances. Even after carefully planned informed consent procedures are carried out, patients may not fully process the nature and implications of the medical research in which they are participating. The research focuses on the question of how level of cognitive functioning and anxiety influence the patient's understanding of disease and treatment in a research setting. Participants are child, adolescent, and adult in-patients and a comparison group of healthy participants. Psychological assessments are standardized tests and interviews.		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project:

Lorah D. Dorn	Social Science Analyst	LDP NIMH
John C. Fletcher	Special Assistant	CC DIR

Project Description:

Participation in medical research is stressful for most patients. Therefore, the required informed consent procedure is executed under relatively stressful circumstances. Even after carefully planned informed consent procedures are carried out, patients may not fully process the nature and implications of the medical research in which they are participating. This study focuses on the cognitive and emotional functioning of children, adolescents and young adults who are hospitalized in a clinical research setting. Specifically, the research focuses on the question of how level of cognitive functioning and anxiety influence the patient's understanding of disease and treatment in a research setting.

Participants in the study are fifteen children (7-12 year olds), fifteen adolescents (13-18 year olds), and fifteen young adults (19-30 year olds) who are admitted to the Clinical Center for the first time. A comparison group of healthy participants matched for age, sex, and socioeconomic status also is included. The socioeconomic background of the participants ranges from working class to professional class. The patients are enrolled in medical protocols involving the treatment of obesity and the treatment of childhood cancer. Level of understanding and reasoning about their illness and treatment regimens are obtained through interviews. Standard tests of cognitive abilities and reasoning about nonstressful content are also administered the second week after admission to the hospital. Anxiety is measured using the Spielberger State-Trait Anxiety Scale. Participants are retested after 6 months. The patient's cognitive functioning when dealing with neutral content is compared with his/her understanding and reasoning about highly personal and stressful medical issues. Level of anxiety is used as a basis for interpreting differences between reasoning about neutral and medical content. This research provides some insights into the informed consent or assent process with children of differing ages, and with children compared with adults.

Significance to Biomedical Research:

This research has clinical significance for informed consent procedures in the conduct of medical research. Information on the relation of anxiety and level of cognitive functioning to patient understanding of research participation can assist investigators in developing more effective methods of communicating complex medical information to participants, especially children. This research has more general theoretical significance for understanding cognitive functioning in highly stressful circumstances.

Proposed Course:

Data have been collected on 32 participants for the first testing period and 10 participants for the second time of testing. Data collection will continue over the next year. Coding is kept current with the data collection. Analysis of data will proceed rapidly once the total sample has been obtained.

Publications:

Susman, E. J., Nannis, E. D., Strope, B. E., Herish, S. P., Levine, A. S., and Pizzo, P. A.: Conceptions of cancer: The perspectives of children and their family. J. of Ped. Psychol. 7: 253-261, 1982.

Nannis, E. D., Susman, E. J., Strope, B. E., Herish, S. P., Levine, A. S., and Pizzo, P. A. Correlates of control in children with cancer and their mothers. J. of Ped. Psychol. 7: 75-84, 1982.

Susman, E. J. Surviving childhood cancer: Social and psychological stresses for children and families. Review of The Damocles Syndrome, Koocher, G. P. and O'Malley, J. E. (Eds.): Contemp. Psychol., 1982, pp. 133-134.

Blumberg, B. D., Lewis, J., and Susman, E. J.: A time of transition. In Eisenberg, M. G. and Jansen, M. A. (Eds.): Impact of Chronic Disabling Conditions on Self and Family. New York, Spring Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02161-03 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Changes in Imitative Learning		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
Leon Kuczynski	Visiting Associate	LDP NIMH
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .20	PROFESSIONAL: .15	OTHER: .05
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 0;"> This study is concerned with the development of children's <u>imitative behavior in natural environment</u>. Data were obtained on 30 children over a nine-month period during the second and third years of life. Sources of data consisted of descriptive accounts of imitation by mothers trained in observational recording. Mothers' records included children's immediate and delayed imitations. Frequently occurring categories included the imitation of positive and negative affective expression, mannerisms and idiosyncratic behaviors of others. A large number of incidents consisted in the imitative practice of instrumental skills and interpersonal social competencies (e.g. household chores, self-care behaviors, affection and caretaking behaviors). Children also imitated aspects of parental disciplinary behaviors both in the context of controlling their own behavior and also when controlling the behavior of others. The implications of these findings for the environmental transmission of adaptive and disordered patterns of behavior are discussed. </p>		

Names, Titles, Laboratory and Institute Affiliations of Other ProfessPersonnel Engaged on the Project:

Carolyn Zahn-Waxler	Research Psychologist	LDP	NIMH
Marian R. Yarrow	Chief	LDP	NIMH

Project Description:

This study investigates the early development of children's imitative behavior in the natural environment. Children's imitation of parents, siblings and other models has been considered to be an important process in children's acquisition of adaptive and maladaptive patterns of behavior. Previous research with preschool and older children suggests that aggressive, prosocial, self controlled and impulsive patterns of behavior may be acquired by imitation. However, the primary focus of research has been on the experimental manipulation of variables such as the characteristics of the model or of the situation that govern the process of imitation in laboratory settings. Information is lacking about what kinds of behaviors children learn through observing models in the natural environment or about the role of imitation in the learning of children in the first years of life.

The present study extends previous research by investigating the development of both immediate and delayed forms of imitation as it occurs in natural settings. One source of data was detailed narrative accounts of children's behaviors recorded by mothers trained in observational procedures. Reliabilities of maternal reports were assessed by comparing mothers' and investigators' reports of children's imitations during home visit (percent of agreement was 91%). Mothers were also interviewed every three weeks during the data collection period and were asked to report new forms of imitation that had occurred since the preceding contact. Data were obtained on 30 children covering a nine-month period in the second and third years of their lives.

Preliminary analyses indicate that during the second year of life imitative repertoires are both extensive and increasingly complex. A large category of behaviors including the imitation of positive and negative affective expressions and idiosyncratic mannerisms and verbalizations of parents and peers implicates imitation as a process in the acquisition of behaviors relevant to the development of expressive personality characteristics. Other sources of frequent imitation served to promote the early practice of instrumentally competent behaviors (e.g. household chores, appropriate use of objects, self-care skills) and interpersonal skills (e.g. caretaking, affection, play, social skills). Children also tended to imitate the verbalizations, tone of voice and behaviors of parents during disciplinary encounters. Children directed their imitations of parental discipline both to themselves and to other persons in the environment. Such imitations, at least initially, may play a role in the acquisition of self-control and in the learning of strategies for influencing and controlling others.

Significance to Biomedical Research

Imitation is a basic process of learning and has obvious implications for the environmental transmission of complex patterns of behavior. An inherent aspect of

the environment of children living with parents suffering from psychopathology is the presence of models of disordered patterns of behavior and affective expression. Although few studies have investigated what behaviors are susceptible to imitation, disordered forms of parental behavior may, in part, be transmitted by this process. This study makes a start in assessing the impact of parent and sibling models by examining the content of imitated behaviors in early childhood.

Proposed Course

The data for this study have been collected. Preparation of a report for publication is underway. The project will then be completed.

Publications

None.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02163-03 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychobiological Correlates of Behavior Problems		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jerome H. Blue Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) National Institute of Neurological and Communicative Disorders and Stroke		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project has been discontinued.		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02164-03 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Impact of Biological Changes on Psychological Functioning During Adolescence		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Elizabeth J. Susman Senior Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) Developmental Endocrinology Branch		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years 3.30	PROFESSIONAL: 1.55	OTHER: 1.75
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This research focuses on psychological development through the <u>pubertal period</u> and on relations between psychological development, <u>biological changes</u> , and parental <u>rearing behaviors</u> . Participants are male and female 9- to 14-year-olds and their parents. At three times of measurement, six months apart, participants are evaluated on psychological and biological variables. Biological measures are stage of <u>pubertal development</u> , assessed by blood samples for <u>gonadotropins</u> , <u>gonadal steroids</u> , and <u>adrenal androgens</u> and by a physical examination to determine pubertal Tanner stage. Psychological measures include assessments of cognitive functioning, self-concept, affective states, interpersonal behaviors, and psychological and behavioral problems. Assessment of parental behavior is through standard inventories regarding child-rearing attitudes and behavior and through observations of parent-child interactions in standard laboratory situations.		

Names, titles, laboratory and institute affiliations of other professional personnel engaged on the project:

Editha Nottelmann	Senior Staff Fellow	LDP NIMH
Jerome H. Blue	Staff Fellow	LDP NIMH
Gale E. Inoff	Research Psychologist	LDP NIMH
George P. Chrousos	Senior Investigator	DEB NICHD
Florence Comite	Clinical Associate	DEB NICHD
Gordon B. Cutler	Senior Investigator	DEB NICHD

Project Description:

Problem and Rationale

Adolescence is recognized as a developmental period characterized by rapid and fundamental biological and psychological change. It is a period in which many psychological problems appear that seriously impair development and behavioral functioning. Although many of the difficulties may have antecedents in earlier periods, the stresses related to puberty are thought to exacerbate these earlier problems and to create new problems and vulnerabilities. The present research addresses questions regarding the role of endocrine and physical growth changes as well as social environmental factors in adolescents' psychological functioning. The conception of psychological development that guides our choice of variables and research design is one in which cognitive, affective, and interpersonal competencies and dysfunctions need to be assessed simultaneously and across time. The objectives of this research are to examine: (a) the interrelations among these psychological processes in early adolescence; (b) the relations between these psychological processes and biological variables (endocrine and physical growth levels and changes); and (c) the interactions of childrearing variables, biological development, and the adolescent's psychological functioning. Each objective is approached cross-sectionally and also longitudinally by following each child over a period of a year.

Methods and Findings

Early adolescence is the developmental period that is the focus of this study. Defined by chronological age, the participants in the study are 9- to 13-year old girls and 10- to 14-year old boys. Participants are defined also in terms of pubertal stage based on Tanner's five-stage criteria. Equal numbers of boys and girls are included at each of the five stages of pubertal development. Approximately 100 adolescents and their parents, working class to professional class, are recruited through a number of religious and secular groups in the Washington area. At three times of measurement, six months apart, the adolescents are evaluated on the biological and psychological measures. A significant problem in assessing the role of biological changes in psychological development has been overcome in this study; namely, the problem of valid and reliable assessment of pubertal status. With the development of radioimmunoassays, it is possible to measure accurately the small amounts of gonadotropins, gonadal steroids, and adrenal androgens that are in the blood and signal the onset of puberty before adolescents or their parents are aware that puberty has begun. Thus, biological assessments include blood levels of gonadotropins, gonadal steroids, and adrenal androgens as well as height, weight, head circumference, and Tanner stage of pubertal development. Parents and adolescents also provide their perceptions of the adolescent's physical development and change. The biological data are collected

as part of this protocol or as part of Protocols #80-CH-32 and #80-CH-160 conducted by the Developmental Endocrinology Branch, NICHD.

Measures of psychological functioning include: cognitive tests of spatial and verbal abilities, assessments of self (self-image, gender identification, and self-esteem), ratings of daily moods and perceived instigations of mood changes, reported relationships and social networks with peers and family, and reported functioning in school. These measures are self-reports. Parallel data are obtained from the parents on the adolescent's moods, relationships in the family and with peers, and functioning at school. Parents also provide a developmental history and provide data on their child-rearing attitudes and practices. Two measures aimed specifically at identifying problem behaviors are the parent's assessment (Achenbach Child Behavior Checklist) and a psychiatric interview with the adolescent. The adolescents and their parents come to the Laboratory, where most of the measures are completed and where parent-adolescent interaction is observed. The parents and child work together in triads and dyads on conflict-resolution tasks (e.g., how to handle family problems and disagreements regarding personal characteristics of the adolescent). The interactions are video-taped and later coded on variables relevant to the study hypotheses.

Very preliminary correlational analyses indicate a number of associations between Tanner stage of pubertal development and level of gonadotropins, gonadal steroids, and adrenal androgens. Preliminary findings from the Achenbach Child Behavior Checklist show that aggressive and undercontrolled behavior problems appear in the early stages of puberty for girls and in the later stages of puberty for boys.

Significance to Biomedical Research:

The study addresses two etiological issues in the development of psychological problems during early adolescence. One issue is the role of biological factors as causes of maladjustment and behavior problems in adolescents. The second issue is the role of child-rearing practices as influences on the adjustment and emotional development of adolescents. Findings will have implications for prevention and intervention programs for adolescents and their families.

Proposed Course:

Data collection is underway, with 90% of the participants evaluated for time one, 40 percent for time two, and 15 percent for time three. Data collection will be completed in 1 1/2 years. Data are currently being coded.

Publications:

Hamburg, B. A., and Inoff, G. E.: Coping with predictable crises of diabetes. Diabetes Care, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02165-01 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Adjustment to Stress in Early Adolescence: Family and Peer Influences		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Editha D. Nottelmann Senior Staff Fellow LDP NIMH		
COOPERATING UNITS (if any) Montgomery County Public Schools		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .80	PROFESSIONAL: .30	OTHER: .50
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This study examines <u>psychological adjustment</u> in the context of family and peer influences in the <u>transitional period of early adolescence</u> . This is a period in which children experience multiple changes and, therefore, are subject to <u>stress and vulnerability</u> . Supportive relationships are hypothesized as important moderators of the effects of stress. Assessments focus on the contributions of <u>family and peer relations</u> and <u>peer/adult networks</u> to children's psychological adjustment and <u>adaptation to change</u> .		

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel Engaged on the Project:

C. Jean Welsh

Psychologist

LDP

NIMH

Project Description:

The study examines psychological adjustment in the context of family and peer influences in early adolescence, a period of considerable stress and high vulnerability. This research has as its primary objective the identification of protective factors, or moderators of stress, in the lives of the adolescent. Personal relationships take on special significance during this transitional period, when children are taking the first steps away from dependence on their parents and beginning to seek psychological support from their peers. Failure to establish and/or maintain supportive relationships may heighten the risk for poor adjustment or serious problem behavior. Assessments focus on the contributions of family and peer relations and peer/adult networks to children's psychological adjustment and adaptation to change.

The participants are 162 eleven- to thirteen-year-old children and their parents. The children were interviewed in their home for two to three hours about their activities and significant relationships. Their parents provided analogous information in a questionnaire. Detailed information was obtained about discontinuities and other important events in the children's lives during the past year; about children's relations with their parents, peer relations, and extrafamilial social networks; about children's participation in group activities; and about children's future goals. These children are a subgroup of the 445 normal volunteers who participated in a study of transition (see Annual Report Z01 MH 02150-04 LDP). Therefore, self-, peer-, and teacher-report data are available on their psychological adjustment in school during the year prior to interview and parent questionnaire administration.

After a normative base is established for the sample on networks, family and peer relations, and children's activities in early adolescence, analyses will profile the lives of children who report low self-esteem, the lives of children who are rated as low on general competence by their teachers, and the lives of children who report aggressive impulses. Analyses also will profile the psychological functioning of children who have problems in school, children who have atypical relations with their parents, children who have atypical relations with their peers, and children who have atypical social networks.

Significance for Biomedical Research

Problem behavior and disordered affect peak in adolescence. The various ecological factors contributing to, or protecting against these difficulties still are poorly understood. This research is expected to yield valuable information on the contributions of family and peer influences on the range of children's psychological functioning.

Proposed Course

Data processing is under way. Manuscripts are in preparation.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02166-01 LDP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Developmental Patterns of Cognition and Interaction in Children at Risk		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
Blaise Pierrehumbert	Guest Worker	LDP NIMH
COOPERATING UNITS (if any) Catholic University, Washington, D.C.		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years 1.5	PROFESSIONAL: 1.3	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) One contribution to maladaptive or aberrant psychological and social functioning of children is distortions in their perceptions of their social, personal, and physical environments and distortions in their causal attributions regarding their responsibility for these events. The aims of this project are threefold: (a) To develop measures of <u>social perspective taking</u> , and of <u>causal attribution of physical, social, and affective events</u> ; (b) To examine <u>longitudinally</u> the evolution of different patterns of social understanding in young children; (c) To examine relationships between social cognition and causal attribution and interpersonal behavior and how these relationships are influenced by developmental processes. Forty-eight children participated when they were approximately two and five years of age. Children's interactions with peers and mother were evaluated for frequency of interaction, complexity of each type of interaction, and initiation. Mothers of all the "target" children have <u>diagnoses of normal or depressed</u> . A second group of children (N = 40; 4 to 11 years) also participated in a social communication problem-solving task. These studies should enhance our understanding of cognitive processes involved in adaptive and maladaptive social behaviors.		
(503)		

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Ronald J. Iannotti	Research Psychologist	LDP	NIMH
David Pellegrini	Assistant Professor	Catholic University	

Project Description:

The accomplishments of young children in understanding, organizing and interacting with the physical and social phenomena in their environments are overwhelming. One of the crucial tasks for developmental psychology is to understand children's perceptions of physical and social events, and their causal attributions regarding responsibility for these events, and to identify relationships between these skills and children's interpersonal behaviors. Therefore, studies have been undertaken which focus (a) on the development of social cognitions, including causal attributions, in children from 2 to 11 years of age, and (b) on links between these processes and social interactions that involve the child with mother and with peer.

A major requirement for successful pursuit of these objectives is the development of measures, suitable for the age levels of the children, of social perspective-taking and of causal attributions of physical, social, and affective events. Procedures have been developed and tested. The procedure for assessing causal attributions for physical events borrows heavily from the methods of Piaget. A variety of physical phenomena are demonstrated and the child is interviewed. A measure of social perspective-taking assesses the child's application of causal attributional processes in personal decisions. A third measure assesses responsiveness and understanding of positive and negative emotional experiences. Additional assessments are made of the child's intelligence and mood state.

In study A, 48 two-year-olds were observed with a familiar peer in play interaction in the laboratory. The mothers of the "target" children have SADS diagnoses of normal or depressed. Three years later the children and their mothers were observed again with a new peer. Interactions were evaluated for social initiatives by the child toward mother and peer, the child's understanding of certain aspects of social interactions, and the complexity of mother-child and peer social interactions. Comparisons of interactions at 2 years and 5 years are made. The cognitive tests described above are administered to the 5-year-olds. The interrelations between these measures and social interaction are examined.

In study B, 20 children with mothers diagnosed as depressed and 20 with mothers diagnosed as normal are studied. The children are between four and 11 years of age. Children are brought to the laboratory in pairs matched for sex and age. One member of each pair is from the group of normal mothers, the other from the group of depressed mothers. The cognitive and mood measures are obtained. Also the pair is given a social communication problem-solving task.

The first aim, development of measures, suitable to these age groups, which successfully differentiate individual patterns of causal attribution across social, physical, and affective domain, appears to have been achieved. Extensive pilot testing and responses to ongoing evaluations of the research samples indicate considerable stylistic differences in causal attribution, perspective taking, and empathy, which are sensitive to the situational and developmental dimensions of interest in this study. Evaluation of the "process" aims awaits completion of the data collection.

Significance to Biomedical Research:

Adaptive and maladaptive interpersonal behaviors in early and middle childhood have cognitive components that have tended to be ignored, while emphasis has been on overt behavior patterns. This research will contribute tools for assessments of cognitive processes and will provide some answers to the complicated issues of cognition-behavior relationships.

Proposed Course:

Data collection is near completion. Analyses have proceeded concurrently. Reports will be in preparation in the coming year.

Publications:

Zahn-Waxler, C., Iannotti, R.J., and Chapman, M.J.: Peers and prosocial development. In Rubin, K.H. and Ross, H.S. (Eds.): Peer Relationships and Social Skills in Childhood. New York, Springer-Verlag, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02167-01 LDP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interpersonal Inferential Abilities in Normal and Depressed Mother-Child Pairs		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Ronald J. Iannotti Research Psychologist LDP NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION Institute and Location: NIMH, Bethesda, Maryland		
TOTAL Staff Years .4	PROFESSIONAL: .2	OTHER: .2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The self-preoccupation and egocentrism sometimes observed in affectively ill adults may significantly interfere with their ability to understand the experiences and needs of other persons. Such a deficit in a parent could seriously interfere with rearing functions. The purpose of this research is (a) to examine <u>role-taking</u> and <u>interpersonal problem-solving</u> abilities of depressed and non-depressed mothers, (b) to examine the development of parallel abilities in their <u>children</u> in the first years of life, and (c) to explore correspondences between parents' and children's capabilities and handicaps in making appropriate inferences about others' psychological states. Forty-eight mothers of two year olds were diagnosed using the SADS procedures and DSM-III criteria. Mothers' abilities to make inferences about others' internal states were assessed in hypothetical social problem-solving situations and in structured interactions with their child. Children's abilities were assessed in both natural and experimental environments. This research will provide information on rearing experiences which either predispose the child to difficulties in interpersonal functioning, or provide the child with training in effective interpersonal skills. Depressed mothers, if deficient with respect to social sensitivities, may provide a pathogenic learning environment.</p>		

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Carolyn Zahn-Waxler, Ph.D.	Research Psychologist	LDP	NIMH
E. Mark Cummings, Ph.D.	Staff Fellow	LDP	NIMH

Project Description:

The self-preoccupation and egocentrism sometimes observed in affectively ill adults may significantly interfere with their ability to understand the experiences and needs of other persons. Such limitations in a parent could seriously impede effective childrearing. If the parent has little awareness of others, including the child, the consequence is likely to be little ability to understand or empathize with the child. Depression sometimes may paradoxically have the opposite effect of heightened sensitivity to others' needs. The purpose of this research is (a) to compare depressed and non-depressed mothers in their capabilities in making appropriate inferences about others' intentions, motives, and feelings and (b) to examine the development of parallel abilities in their young children. Comparisons of children of depressed mothers who are sensitive in interpersonal problem-solving abilities with those who are not may aid in understanding why some children with an affectively ill parent show adequate psychosocial adjustment while others develop behavior problems.

Methods:

Forty-eight mother-child pairs were studied. Children were 2 to 2-1/2 years of age. Mothers were given psychiatric interviews (SADS). DSM-III criteria were used to identify mothers with major depression and minor depression and mothers who were normal. Parental abilities to make inferences about internal states of others were assessed in standard hypothetical social problem-solving dilemmas and in structured interactions with their child.

The children's cognitive (spatial, social, and affective) awareness was assessed in both natural and experimental environments. Experimental contexts were designed to elicit a range of interpersonal functioning (e.g., responding to a familiar peer who is distressed by the absence of his/her mother or responding to two adult strangers in a verbal argument). Children's reactions are evaluated for evidence of a child's social sensitivities.

Significance to Biomedical Research:

The present research will provide insight into the processes by which adaptive and maladaptive social functioning is transmitted to children. It will provide information on rearing experiences which either predispose the child to difficulties in interpersonal functioning or provide the child with training in effective interpersonal skills. Depressed mothers, if deficient with respect to social sensitivities, may provide a pathogenic learning environment.

Proposed Course:

Data analyses have just begun on the completed data-gathering phase of the research. A year of analysis and preparation of reports is projected.

Publications: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02168-01 LDP
PERIOD COVERED November 6, 1982 through July 7, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Socialization in Early Infancy: Patterns of Interaction in Two Cultures		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Yorio Kosawa Guest Worker LDP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology, NIMH		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years: 1.01	PROFESSIONAL: 1.00	OTHER: .01
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The effect of the infant's <u>temperament</u> on its <u>caregiver</u> is investigated. One study involves investigation of <u>maternal</u> and <u>infant behaviors</u> in an <u>American middle class sample</u> at one and three months of age, in order to further compare with the <u>Japanese data</u> obtained by previous researchers and also to understand how infant's temperament affects <u>maternal behavior</u> in early infancy. A second study is designed to investigate the nature of <u>peer</u> and <u>teacher-infant interaction</u> during the second and third quarter of the second year and the first half of the fourth year in the <u>day care situation</u> and to monitor these changes according to the <u>developmental stage</u> of the infant.		

Names, titles, laboratory and institute affiliations of professional personnel engaged on the project:

Marian R. Yarrow	Chief	LDP NIMH
Howard A. Moss	Guest Worker	LDP NIMH

Project Description:

Since 1960s the explicit recognition has been widespread that children have an effect on their environment as well as vice versa. According to this recognition several studies have been conducted to verify how the infant's temperament influences his/her caregiver's behavior in an interactional process.

The purpose of the research is to examine infant development in relation to differences in culture and temperament. Caudill and Weinstein (1969) conducted a comparative study of mother and infant behaviors in America and Japan. Their results showed that American mothers emphasized distal behavior toward their infants and that the infants tended to be active and vocal, whereas Japanese mothers tended to have more close physical contact with their infants who in turn were rather quiet even during the awake state. Caudill concluded that maternal behavior was influenced by the culture and that this influenced the infant behavior at a very early stage in life.

There is, however, another interpretation of Caudill's result if we find temperamental differences of infants in both cultures. That is, American infants' temperament tends to make their mothers behave in a distal way, whereas Japanese infants' temperament tends to make mothers contact closely.

In his study, Kosawa (1982) collected data of mother-infant interactions at one and three months of infant's age and also assessed the individual responsiveness of the infants by administering the temperament assessment. He found in his Japanese sample that mothers' behaviors toward female infants, but not males, were influenced by temperament. This effect on maternal behavior is stronger at three months than at one month. In his result, age, sex and temperament of the infants seem to be important parameters in addition to culture.

Two studies have been undertaken. One study investigates maternal and infant behaviors at one and three months of age in order to further compare the Japanese data obtained by Caudill and Kosawa and also to understand how infants' temperament affects maternal behavior in early infancy. A second study is designed to investigate the nature of peer and teacher-infant interactions between 14 and 18 months and 36 to 42 months in the day care situation and to monitor these changes according to the developmental stage of the infant.

Study I Observational mother-infant study.

Twenty mothers and infants are observed and video-taped at their homes for two and a half hours in the morning and for two and a half hours in the afternoon. Home visits are scheduled for when the infant is one month (+4 days) and three months (+4 days) of age. The video equipment is placed at least six feet from the infant. The investigator moves the equipment as is necessary in order to follow the mother and infant. When the video-taping is finished at the three-month visit, the mother is asked to fill out the Carey Temperament Scale.

Study II Observational toddler study in day care situation.

Fourteen to eighteen month old infants and thirty-six to forty-two month old infants are selected for observation and video-taping. The video-taping scans the entire class and from time to time focuses on person to person interactions. A designated sampling procedure is used to ensure the use of the same procedures on each visit. Taping is done for one two-hour session each week. The video camera is put in the corner of the class room. However, occasionally the equipment is moved from place to place to facilitate the taping.

Significance to Biomedical Research:

Nature-nurture controversy is the old but recent problem. The goal of this research is to understand the interaction of disposition and environment in the infant development. Identification of these factors facilitates planning early preventive strategies and interventions in adult-infant interaction.

Proposed Course:

Data collection is completed. Analyses will be completed in the coming year.

Publications:

NONE

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02169-01 LDP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interactions between siblings with a depressed parent		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Carolyn Zahn-Waxler Research Psychologist LDP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL Staff Years .20	PROFESSIONAL: .20	OTHER: 00
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purpose of this research is to examine contributors to systematic <u>differences in siblings</u> from different families in the degree to which their relationships with each other are adaptively or maladaptively focused. Some brothers and sisters fight frequently and chronically while others get along and take care of each other. It would be important to learn some of the reasons why this is so. Siblings with normal and depressed mothers are compared. If a mother is depressed and less able to care for her children some of the responsibility for <u>caregiving and mediation of conflicts</u> may fall to the older sibling. This would have implications for the <u>psycho-social development</u> of both the older and the younger sibling. Siblings are observed in interaction alone with each other, individually with the mother and together with the mother. The younger child is between the ages of two and three and the older child is between the ages of five and seven. Coding systems are currently being developed in which episodes of caregiving and conflict are identified and analyzed in relation to <u>maternal psychopathology</u>. </p>		

Names, titles, laboratory and institute affiliation of other professional personnel engaged on the project:

Dale Hay	Guest Worker	LDP NIMH
Marian Radke-Yarrow	Chief	LDP NIMH
Sarah Friedman	Research Psychologist	LDP NIMH

Project Descriptions:

Early psychoanalytic conceptualizations of interpersonal relations and affective communication patterns between siblings focused principally on the jealous nature of sibling interactions. More recent anthropological research has emphasized the adaptive significance of siblings in the socialization process: That is, relationships between siblings are characterized by nurturance and caretaking as well as by jealousy and aggression. And ethologists too (e.g., Hinde) define the sibling system as one in which the potential for both altruism and aggression is heightened. The purpose of this research is to examine contributors to systematic differences in siblings from different families in the degree to which their relationships with each other are adaptively or maladaptively focused. Some brothers and sisters fight frequently and chronically while others get along and take care of each other. It would be important to learn some of the reasons why this is so. Siblings with normal and depressed mothers are compared. If a mother is depressed and less able to care for her children some of the responsibility for caregiving and mediation of conflicts may fall to the older sibling. This would have significant implications for the psycho-social development of both the older and the younger sibling.

Method and Results:

In a laboratory setting designed to closely approximate the natural rearing environment (see Z01 MH 02144-03), siblings are observed in interaction alone with each other, individually with the mother, and together with the mother. The younger child is between the ages of two and three and the older child is between the ages of five and seven. In this study, unlike many other studies of parent-child interaction, the family members are not only observed while playing together, but while trying to accomplish other things: The mother must serve a snack, the older sibling must watch over the younger while the mother is out of the room, the children must each attempt to solve challenging and potentially frustrating problems, and the family must reunite after the children have had separate stimulating experiences.

To tap the harmonious and disharmonious dimensions of family relations in these differing contexts, two general types of interactions are being examined: (1) caregiving episodes in which one family member (mother, older sibling, or younger sibling) meets an explicit or implicit need of another, and (2) conflict episodes in which one family member protests, resists, or retaliates against another person's action, i.e., situations in which their needs clash. These two types of episodes will reveal the social skills possessed by all three family members (e.g., role-taking, abilities to negotiate solutions to interpersonal problems, etc.), and they will offer opportunities for the display of prosocial and aggressive actions. Furthermore, the patterning of caregiving and conflict episodes, as well as the content and affective quality of the particular actions taken by the siblings and the mother, may be used to characterize each person's interactive style, and may be analyzed in relation to maternal psychopathology.

Preliminary empirical observations indicate that the quality of sibling interaction varies markedly across families; further, there is reason to believe that the patterns of harmonious and conflictual interactions between siblings are linked to maternal patterns of caregiving (but not always in a uniform manner). For example, some older siblings who give sensitive care to the younger child have been themselves observed to experience high levels of nurturance and competence from their mothers. The other extreme can also be seen in cases where mothers' maladaptive patterns of caregiving toward the older child (e.g., derision, sarcasm, and belittlement) are replicated in the older sibling's treatment of the younger child. Older children are also observed to "fill in" for the mother, e.g., finishing mother's sentences for her and competently taking charge in situations where the mother has difficulty. The older sibling's caregiving, however, seems to depend on characteristics of the younger sibling, such as prevalent mood, latency to anger, mastery motivation, strivings toward maturity, etc., which in turn may be linked to maternal pathology.

Significance to Biomedical Research and the Program of the Institute:

The ability to competently and empathically take care of others, both within and outside the family, is considered to be a hallmark of mental health (Spitzer & Endicott). It represents the other side of mental illness, which characteristically requires being cared for by others. Factors contributing to positive and negative caregiver capacities may be better understood by studying the phenomenon from a developmental perspective in families with and without emotionally disturbed caregivers.

Proposed Course

A coding system is currently being developed to characterize conflict and caregiving episodes between siblings. Codes are being developed that can be used across the differing interactive contexts but that will also be sensitive to the unique demands of each challenge the family members face. Coded data will then be analyzed and prepared for publication.

Publications:

Zahn-Waxler, C.: Review of, The young child: Reviews of research, (Vol. 3). In Moore, S. G. and C. R. Cooper (Eds.): Contemporary Psychology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02170-01 LDP
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychiatric Evaluation of Infants and Toddlers		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Leon Cytryn Research Psychiatrist LDP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Developmental Psychology		
SECTION		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland		
TOTAL Staff Years .26	PROFESSIONAL: .25	OTHER: .1
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Diagnostic instruments</u> for the <u>psychiatric evaluation</u> of infants and toddlers were developed, tested, and used. The subjects are 2 to 5 year-old-children of depressed and normal mothers seen in a project designed to study the <u>environmental factors</u> underlying the <u>transmission</u> of <u>affective</u> from parent to child. The instruments used in the initial evaluation of these children were a semi-structured play interview consisting of 3 10-minute segments: free play, doll play, and aggressive play. These interviews plus Achenbach Inventory plus a social history of the child were used to make a DSM III diagnosis or to describe various areas of concern. </p> <p> At present a new rating scale is being devised and tested for use with current subjects plus younger children, down to 1-1/2 years of age. </p>		

Names, Titles, Laboratory and Institute Affiliations of Other Professional Personnel Engaged on the Project:

Donald McKnew	Research Psychiatrist	LDP	NIMH
---------------	-----------------------	-----	------

Project Description:

Reliable diagnostic instruments for use with young children 1-1/2 to 5 have not previously been available. In the past, affective illness in latency age children, adolescents and infants has been given much attention. However, most studies have bypassed the toddlers (ages 1-1/2 to 5 years). Although it may not be possible to make exact diagnoses at this age, one would expect to find disturbances which might conceivably be precursors of future psychopathology. Finding of such precursors would a) contribute to the developmental view of affective illness and b) could be crucial to any attempts of secondary prevention.

The toddlers who are being evaluated are part of a large childrearing project in which the children of 4 groups of mothers are being studied (major depression, bipolar disorder, minor depression, and normal) (see Project Z01 MH 02156-04).

1. A semi-structured psychiatric play interview was developed to interview children 2-5 years of age. The interview consists of 3 10-minute segments: free play with neutral toys (blocks, crayon and paper, ball, doll, teddy bear), doll house play with small human figures whose age and sex duplicate the true family constellation, aggressive play with guns, soldiers, boxing gloves, punching bag, and a pounding block.

Before the testing begins, separation from the mother is observed and noted. In the first segment, rapport is established and the child is encouraged to use the toys in any manner he or she chooses. In segment two, the child is encouraged to play out various family events such as meals, parties, bedtime, bathroom or kitchen scenes. However, as in segment one, the child remains free as to his or her activities. In the 3rd segment, the child is encouraged to use all the various aggressive toys with or without the examiner's participation. Despite the encouragement, the child again is free to do as he wishes. Running notes are kept by the examiner or by an observer through a one-way mirror and each session is recorded on video cassettes. Following the interview, 4 rating scales and the mental status section of the CAS are filled out. The first scale concerns the process of separation from the mother. Then each interview segment is rated separately, using the same rating scale employed in other parts of the rearing study.

Significance to Biomedical Research:

With the increasing awareness of the frequency of diverse psychopathological states in infancy and childhood, the need for reliable and valid diagnostic instruments in the toddler age group is increasingly apparent. Such an instrument, in addition to providing a useful tool for research, has also the potential for use in clinical settings for diagnosis, prevention, and treatment.

Proposed Course:

The interviews are in use in all phases of the Rearing Study with both infants and toddlers.

Publications:

Cytryn, L.: Personality development in patients with Down's Syndrome receiving 5-hydroxytryptophan or placebo. In Colman, M., Barnett, A., Lodge, A., and Cytryn, L. (Eds.): Serotonin in Down's Syndrome. Amsterdam, North Holland Publishing Co., 1973, pp. 87-93.

Gaensbauer, T.J., Harmon, R.J., Cytryn, L., and McKnew, D.H., Jr: Social-affective development in infants of manic-depressive parents. Am. J. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 201 MH 00478-27 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neural mechanisms of memory and habit formation		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Mortimer Mishkin, Chief, LN, NIMH		
COOPERATING UNITS (if any) Howard University University of North Carolina Towson State University		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 5.5	PROFESSIONAL: 2.0	OTHER: 3.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>anterior temporo-insular cortex</u> in the <u>macaque</u> consists of the highest-order sensory processing stations for all the sensory modalities. We have proposed that this cortex contains the <u>stored representations of stimuli</u> to which the organism has been exposed. The storage is the result of activation by anterior temporo-insular neurons of a <u>limbo-thalamic pathway</u>, which actually consists of two parallel pathways, one involving the <u>amygdala</u> and the magnocellular portion of <u>nucleus medialis dorsalis</u>, and the other involving the <u>hippocampus</u> and the <u>anterior thalamic nuclei</u>. <u>Recognition memory</u> occurs when the stored representation of a past stimulus is reactivated by a current stimulus, and <u>associative memory</u> occurs when that stored representation is linked to the stored representation of another stimulus or another event, such as a location, an emotion, or a motor act. These various types of memory can be distinguished from <u>habits</u>, which appear to be independent of the limbo-thalamic system. </p>		

Principal Investigator:

Mortimer Mishkin Chief, Laboratory of Neuropsychology LN NIMH

Other Investigators:

J.P. Aggleton	Visiting Fellow	LN NIMH
E.A. Murray	Staff Fellow	LN NIMH
J. Bachevalier	Guest Worker	LN NIMH
B.H. Turner	Professor	Howard University
W.H. Overman, Jr.	Asst. Professor	Univ. N. Carolina
H. Petri	Chairman, Psychology Dept.	Towson State Univ.

Technical Support Staff:

L. Cali	Biological Aid	LN NIMH
L.I. Dorsey	Biological Lab. Tech. (Animal)	LN NIMH
R.R. Phillips	Psychology Technician	LN NIMH
L.P. Stokes	Biologist	LN NIMH

PROJECT DESCRIPTION:

Objectives:

The objective of the studies in this project is to delineate the neural system underlying memory formation in the monkey and to differentiate it from the neural system that underlies habit formation. The methods used include behavioral analyses of the effects of selective cerebral ablations and disconnections combined with anatomical analyses of functional neural pathways. The rationale and design of the studies are often based directly on information derived from other projects in this laboratory, many of which deal with the pathways for, and, mechanisms of stimulus processing and encoding. The results from these other projects suggest that the sensory system for each modality is composed of two hierarchically organized corticocortical pathways, one directed ventrally to the temporal-lobe limbic system and concerned with object perception, the other directed dorsally to the frontal-lobe motor system and concerned with spatial perception. The ultimate goal of this project is to determine how object and spatial perceptions in the different modalities are formed into memories, how these different memories are associated with each other, how they evoke emotions and motor acts, and how they lead not only to these cognitive events but also to habit formation. Our progress in understanding each of these processes will be described in turn.

(1) Recognition memory:

Previous studies suggested that one-trial object recognition (delayed nonmatching-to-sample with trial-unique objects) depends on a reciprocal cortico-limbo-thalamic pathway that leads to the storage of the encoded representation of the stimulus in anterior temporo-insular cortex. New studies have shown that this pathway actually contains two relatively independent limbo-thalamic segments, one from the amygdala through the amygdalofugal pathways (AFP) to the magnocellular portion of n. medialis dorsalis (MDmc), and the other from the hippocampus through the fornix (Fx) to the anterior thalamic nuclei (Ant N). The evidence is based on comparison of the effects of separate and combined AFP and Fx transections, as well as of separate and combined MDmc and Ant N ablations. In both cases, the combined lesions yielded significantly greater recognition losses than did the separate lesions. These recent results resemble our original finding that combined removal of the amygdaloid complex (A) and hippocampal formation (H) yielded far greater recognition losses than did their separate removal. Neither the A lesion nor the H lesion alone, however, had included substantial portions of the entorhinal cortex (Ent), whereas the combined lesion had included all of it. The original results were therefore also consistent with the possibility that the severe recognition loss was attributable to either H+Ent or A+Ent lesions. A test of this possibility has now shown that H+Ent lesions yield about the same mild effect as hippocampectomy alone, whereas A+Ent lesions yield a severe deficit comparable to that following the combined A+H lesion. Thus, the Ent lesion appears to be equivalent to a hippocampectomy in this situation, presumably because it disconnects the hippocampus from the inferior temporal visual cortex. The results support the earlier conclusion that combined damage to the amygdaloid and hippocampal systems is necessary to produce a profound recognition loss in monkeys, a loss that has now been seen in both vision and touch.

Because of their newly discovered functional significance, we undertook to map the two limbo-thalamic projection systems in detail using axonal transport techniques. The results show that the amygdaloid projections arise throughout the complex, though most heavily from the basomedial nucleus, sweep through the substantia innominata, and then travel in the inferior thalamic peduncle to enter the head of the thalamus before passing caudally to terminate in MDmc and n. reuniens; allocortical areas adjacent to the amygdala also contribute significantly to this projection. Hippocampal projections arise predominantly in the subiculum and terminate most heavily in nuclei anterior medialis, anterior ventralis, and lateralis dorsalis, with lighter projections to nuclei reuniens, centralis latocellularis, rotundis, and paraventricularis; all of these projections course through the medial part of the fornix, though n. lateralis dorsalis also receives a nonfornical input which runs through the medial pulvinar.

In view of the importance of the two limbo-thalamic pathways for recognition memory, we are now trying to determine whether their prefrontal targets might also be involved in this function. To examine this, we have prepared animals with combined lesions of ventral prefrontal cortex (the target of MDmc) and

adjacent anterior cingulate cortex (the target of Ant N). The performance in visual recognition memory of these animals with ventromedial (VM) lesions are being compared with those of animals given dorsolateral (DL) lesions of the prefrontal cortex. The results thus far indicate that the VM lesion produces a severe impairment in recognition, whereas the DL lesion produces almost none. Comparison of these new results in memory with those obtained many years earlier on spatial delayed response suggests that: (i) presumably, because of its connections with the limbo-thalamic pathways, ventromedial prefrontal cortex (about which there has been little functional information until now) may be more important than dorsolateral prefrontal cortex in general memory processes and (ii) the classical delayed-response deficit after dorsolateral prefrontal lesions may represent a perceptual-mnemonic impairment in spatial functions rather than a strictly mnemonic one.

(2) Associative memory:

Like their efferent pathways and thalamic targets, the amygdala and hippocampus make approximately equal contributions to recognition memory. In the case of associative memory, however, new results indicate that these two limbic structures make very different contributions. In one experiment, monkeys were trained preoperatively on a visual recognition task and, separately, on a tactual recognition task, with the same set of objects comprising the stimuli for both modalities. One group of monkeys then received amygdalectomies and the other, hippocampectomies, after which both were retrained on the intramodal memory tasks to a high level of performance. When tested later for their ability to perform the recognition task across modalities, i.e. to choose between two visual stimuli after one had been presented as a tactile sample, the hippocampectomized monkeys continued to perform at a high level, but the amygdalectomized monkeys fell to chance performance. Nearly the opposite results were obtained in a second study that tested the ability of monkeys to remember the spatial location of visual objects. In this case, monkeys given amygdalectomy were able to regain the level of performance they had achieved preoperatively, whereas those given hippocampectomy failed to rise above chance. The results of these two complementary experiments indicate that although both the amygdala and hippocampus are important for associative memory, their roles are totally different. Many further analyses along the lines of these two experiments are needed, however, before the selective associative memory functions of the amygdala and hippocampus can be precisely identified. For example, the association of an object with an affective state, such as fear, pleasure, etc. appears to depend much more heavily on the amygdala than on the hippocampus. New support for this view is being obtained in an experiment showing that one-trial object-reward association is impaired more by amygdaloid than by hippocampal lesions (although neither deficit approaches in severity the one produced by combined removal of these two structures). By contrast, because of the contribution to spatial memory that is made by the hippocampus, the association of objects with spatially directed motor acts could depend more heavily on the hippocampus than on the amygdala. Studies to examine this possibility are being planned.

(3) Habit Formation:

On all of the memory tasks described, the deficits are especially severe when removals of the amygdala and hippocampus are combined. Yet, even the combined limbic lesion does not affect all forms of learning and retention. For example, despite their rapid forgetting in one-trial object recognition, animals with the combined limbic lesions have no difficulty learning object discriminations, at least in the standard situation where trials are repeated 3-4 times per minute. In an attempt to resolve this discrepancy between rapid forgetting and successful learning, we tested whether object discrimination learning would be prevented in animals with limbic lesions if intertrial intervals exceeded the putative memory span. Surprisingly, animals with the combined amygdalo-hippocampal lesions learned to discriminate a long list of object pairs even though the list was presented only once every 24 hours. Thus, although the operated animals have an extremely short memory span, they can retain and accumulate information gained from single discrimination learning trials separated by 24-hour intervals. This paradoxical success in the presence of severe memory loss implies the existence of an important retention mechanism outside the limbic structures of the temporal lobe.

We have now performed experiments to characterize further the essential difference in function between the limbic and nonlimbic retention mechanisms. Our results suggest that the limbic system is critical for high levels of retention of object-reward associations after a single acquisition trial with short lists of objects, or after two or three repetitions with long lists of objects but short intertrial intervals. With greater repetition, however, retention of object-reward associations can be mediated in the absence of the amygdala and hippocampus, and the retention appears to be independent of both list length and delay. To distinguish this form of retention from memory, we have labelled it 'habit formation'. Further investigation of this mechanism of habit formation as well as elucidation of its neural substrate have become important goals of our research. For example, preliminary evidence regarding the effects of damage to inferior temporal cortex (area TE) indicates that this cortical region is important not only for the limbic memory system but also for the nonlimbic habit system.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In the process of investigating the role of various temporal-lobe structures in the visual memory of the monkey, we obtained a result that is particularly exciting because it appears to solve the long-standing puzzle concerning the neuropathology underlying the syndrome of global amnesia in man. This syndrome, which is characterized by a profound inability to remember new experiences, has been attributed in the clinical literature to destruction of the hippocampus. Yet, attempts to duplicate this syndrome in monkeys by removal of the hippocampus alone have largely failed. What we have found in our studies, for both recognition memory and associative memory, is that if damage to the hippocampus is combined with damage to the amygdala then a profound memory loss does ensue. The discovery has not only resolved the discrepancy between clinical and animal findings but has also provided new

insight into the neural substrate of memory. Specifically, it has led to the development of a hierarchical model of recognition and associative memory involving a cortico-limbo-thalamic memory circuit that may well serve as the foundation for all cognitive processes beyond perception, including thought. As we gain further understanding of the memory system, and how it differs from the noncognitive system for habit formation, we will inevitably gain a better understanding of thought and its breakdown in normal and abnormal behavior.

PROPOSED COURSE OF RESEARCH:

Since combined removal of the prefrontal cortical targets of the magnocellular portion of n. medialis dorsalis and the anterior thalamic nuclei produced such a severe loss in visual recognition memory, we shall look next at the effects on memory of damaging these two fields separately. Also, having now found severe recognition losses in both vision and touch after lesions of the limbo-thalamic system, we shall look next at the effects of such lesions on auditory recognition, to determine whether the system is indeed critical for recognition in all modalities. In addition, we shall continue our attempts to differentiate between amygdaloid and hippocampal contributions to associative memory and test whether the distinction is carried further through the thalamic and prefrontal segments of the circuit. We shall also initiate studies to explore the neural basis of habit formation, with the cortico-striatal projection system as our initial target.

PUBLICATIONS:

Aggleton, J.P. and Mishkin, M.: Visual recognition impairment following medial thalamic lesions in monkey. Neuropsychologia 21: 189-197, 1983.

Aggleton, J.P. and Mishkin, M.: Memory impairments following restricted medial thalamic lesions in monkeys. Exp. Brain Res. (in press) 1983.

Aggleton, J.P. and Mishkin, M.: Projections of the amygdala to the thalamus in the cynomolgus monkey. J. Comp. Neurol. (in press) 1983.

Mishkin, M., Malamut, B., and Bachevalier, J.: Memories and Habits: Two neural systems. In McGaugh, J.R., Lynch, G. and Weinberger, N.M. (Eds.): The Neurobiology of Learning and Memory. New York, Guilford Press, 1983 (in press).

Mishkin, M. and Petri, H.L.: Memories and habits: Some implications for the analysis of learning and retention. In Butters, N. and Squire, L. (Eds.): Neuropsychology of Memory. New York, Guilford Press, 1983, (in press)

Murray, E.A. and Mishkin, M.: Severe tactual memory deficits in monkeys after combined removal of the amygdala and hippocampus. Brain Res. (in press) 1983.

Zola-Morgan, S., Squire, L.R., and Mishkin, M.: The neuroanatomy of amnesia: Amygdala-hippocampus vs temporal stem. Science 218: 1337-1339, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02032-07 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neural coding of visual stimuli in the awake monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Barry J. Richmond, Medical Officer (Neurology), LN, NIMH		
COOPERATING UNITS (if any) Laboratory of Sensorimotor Research, NEI		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.5	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The inferior temporal cortex is important for the visual processing underlying <u>pattern recognition</u>, and <u>single neurons</u> in this area are responsive to visual patterns. We are studying two aspects of these neuronal responses: one is the role of different <u>stimulus features</u>, and the other is the <u>behavioral context</u> in which the stimulus is presented. The presence of visual stimuli other than the probe stimulus markedly reduces neuronal responses to the probe, revealing an interaction within the visual system. <u>Selective attention</u> to the location or intensity of a probe stimulus will also reduce neuronal responses to it while selective attention to the pattern itself leads to increased neuronal response to the same stimulus. Using powerful new techniques developed for this project, we have found that different <u>2-dimensional pattern features</u> are encoded in different aspects of the <u>time sequence of neuronal discharge</u> both in inferior temporal cortex and <u>primary visual (striate) cortex</u>. The spatial features encoded in different aspects of the neuronal discharge can be reconstructed. </p>		

Principal Investigator:

B.J. Richmond	Medical Officer (Neurology)	LN NIMH
---------------	-----------------------------	---------

Other Investigators:

L. Optican	Senior Staff Fellow	LSR NEI
H. Spitzer	Visiting Fellow	LN NIMH
M. Mishkin	Chief, Laboratory of Neuropsychology	LN NIMH

Technical Support Staff:

M. Podell	Psychologist	LN NIMH
-----------	--------------	---------

PROJECT DESCRIPTION:

Objectives:

One major role of the primate visual system is to extract information from the image falling on the retina regarding the form and nature of visual objects. In the human and presumably in other primates this extraction is manifested as visual perceptions which eventually give rise to visual memories. In order to accomplish these tasks of perception and memory, information about retinal images must be analyzed and transmitted throughout the extent of the visual pattern system. Based on results obtained largely in this laboratory, much of the visual pattern system is presumed to be cortical, starting in striate cortex and ending in the highest order purely visual region known at this time, inferior temporal cortex.

Using single-neuron recording techniques in the awake monkey, we studied two aspects of visual processing. One concerns the role of attention on processes related to pattern vision. The other has entailed developing a new approach to study the representation of visual information in the neuronal spike train.

Ablation studies have shown that inferior temporal cortex is critical for the performance of pattern discrimination and pattern recognition tasks in primates. Furthermore, single-unit recording studies of the anterior part of inferior temporal cortex (area TE) over the past 15 years have shown that many of the visually responsive neurons in this area are strongly activated by visual stimuli with complex features, e.g. a bottle brush, a hand, a face, etc. These recording studies, which were largely carried out in lightly anesthetized, paralyzed monkeys, demonstrated not only that area TE cells are sensitive to particular patterns, but also that the receptive fields of the cells all cover the central part of the visual field, with greatest sensitivity occurring at the center of gaze, or fovea. The receptive fields were also found to be large and to extend into both the contralateral and ipsilateral visual fields.

Other studies, carried out in awake monkeys performing visual tasks, have shown not only that the pattern of the stimulus influences the responsiveness of the single neurons, but also that the part the pattern plays in the task is influential. For example, when a pattern is shown to a monkey so that he can later indicate whether a second pattern is the same as the first or not - delayed matching-to-sample - the neuronal response to the pattern is often weak or absent during the first presentation but strong when the pattern is presented the second time. In order to specify the visual information to which inferior temporal neurons are sensitive, it is first necessary to understand and control these behavioral and attentional influences on inferior temporal neurons, and it is these influences to which our initial experiments have been directed.

Major Findings:

In the attentional experiments, we have so far recorded from over 450 cells distributed throughout area TE of 5 different monkeys. In our initial experiments we unexpectedly found inconsistent neuronal responses to slits and squares of light presented as probe stimuli while the monkey was fixating a smaller spot of light - the fixation spot - in order to detect the dimming of this smaller spot and thereby obtain a reward. In previous studies of inferior temporal neurons, whether carried out in anesthetized or awake monkeys, there was no fixation spot. In order to overcome any possible influence of the fixation spot, we turned it off for a short period (1 sec). If a probe stimulus was presented during this "blink" of the fixation spot, about 60% of the cells encountered gave a highly consistent response. The animal's eye position was monitored automatically, and, if the eye moved more than 1 degree in any direction, the trial was discontinued and the animal had to initiate a new trial. By changing stimulus parameters and stimulus positions, the following results were obtained. In most visually responsive inferior temporal cells, the fixation spot exerts a very potent influence, more potent even than changes in the features of the probe stimulus (although in these experiments only the size and relative proportions of slits and squares were varied). When the same stimulus that caused a neuronal response during the "blink" was presented while the fixation spot remained on, the response was either much weaker or disappeared completely. As a result, the size of the receptive field became much smaller when the fixation spot was present than when it was absent. Under both conditions, however, the best response always occurred when the stimulus was presented at the center of gaze, i.e., projected onto the fovea. The response latencies to stimulus onset were between 70 and 200 msec. These and other characteristics of the neuronal responses to the stimulus during the "blink" of the fixation spot were similar to responses obtained previously by others in the lightly anesthetized, immobilized preparation.

In order to determine if increased attention to the stimulus during the "blink" of the fixation spot might be causing the increased strength and consistency of response, we altered the task so that the monkey, while still fixating, was required to respond to the dimming of the probe stimulus rather than of the fixation spot. When the monkey responds correctly in this

condition, we can assume that his attention must be directed to the stimulus. These experiments were carried out in both the "blink" situation (fixation spot absent) and the fixation situation (fixation spot present). In both cases, the neuronal response to the stimulus was weaker than before. Indeed, in the task in which the monkey responded to the stimulus dimming and the fixation spot remained present, the neuronal response was weakest of all, often to the point of no response, showing that the suppression due to the fixation point and suppression due to attention are additive. Thus, responses of inferior temporal neurons to visual stimuli are suppressed simply by the physical presence of the fixation spot. Furthermore, the improvement of response when the fixation spot is absent cannot be due to a shift in spatial attention to the probe stimulus, because in tasks where such a shift in spatial attention was explicitly required the strength of the response was actually weakened.

This suppressive effect of attention, however, was obtained in behavioral tasks in which the specific pattern of the stimulus was not relevant for correct performance. Since pattern discrimination depends so critically on the integrity of the inferior temporal cortex, we modified the behavioral tasks so that we could compare the results of the foregoing experiments with those obtained when the pattern of the stimulus was relevant for correct performance. In the modified task the monkey was required to distinguish between a simple pattern, such as a plus sign, and the square or slit that had been presented in the previous experiments. The behavioral response to the plus sign was an immediate lever release, while the behavioral response to the probe stimulus, i.e. the square or slit, was to release the lever only when it dimmed. Thus, the behavioral response to the probe stimulus (the slit of light) was in every way the same as it had been in the previous experiments except that the monkey now had to distinguish the shape of the stimulus when it first appeared in order to make the correct response. As in previous tasks, the retinal locus of the stimulus was closely controlled by the requirement that the monkey maintain constant eye position. Many neurons now gave a strong response to the probe stimulus even when the fixation spot (previously a potent suppressive influence) was present. Under these conditions, the strength of response approached or even surpassed that seen when neither suppressive influence was present (i.e. the blink task).

These results show for the first time (a) that attention to the spatial location or intensity of a stimulus or both are not the only attentional factors that influence the responses of single neurons, and (b) that the responses of many inferior temporal neurons may be markedly altered depending on which stimulus feature the monkey is currently attending to. Indeed, attention to different stimulus variables can have opposite influences on the response of the same neuron.

In order to understand what information has been changed due to the attentional effects described above, we must know how the visual information is represented by the neuron. For the past 20 years or so the assumption has been made that the information is encoded in some single function of the firing rate - usually modeled as the mean firing rate over some period

(usually short but not always). Because this assumption has never been truly tested, and because knowing how information is actually encoded is so critical, we have developed a method which makes no a priori assumption except that the sequence of spikes over time is important (which includes the mean firing rate as one possibility). We first studied single neurons in inferior temporal cortex, because previous experiments (see introduction) indicated that these neurons might be very selective for patterns and so might possess complex encoding mechanisms.

In these experiments we chose visual stimuli which can be used to reconstruct completely any complex visual image (mathematically, an orthogonal set of 2-dimensional basis patterns based on Walsh functions). This set of 64 2-dimensional patterns, each 40 degrees across and centered on the fovea, was presented one at a time during the "blink" paradigm described earlier. The fixation light blinked off 300ms before the 400ms stimulus presentation; 400-700ms later the fixation point returned and dimmed. Only successfully completed behavioral trials were analyzed. The poststimulus spike train was then converted into continuous probability functions of spike occurrence (by convolution with a Gaussian pulse) for each of the 64 stimuli. Inspection of these 64 responses revealed that several of the temporal waveforms were different from the others. In order to quantify these differences, the temporal modulation caused by each pattern was decomposed into its principal components with a Karhunen-Loeve (K-L) transform of all the data. This method of analyzing the spike train has three very desirable features. The coefficients of the principal components are independent, so that no information is duplicated in any two of the components. Also, the coefficients are ordered so that the greatest energy of the signal that can be projected onto any component is in the first component, the next greatest energy is in the second, and so on. Finally, the principal components represent the data in the time domain so the variation in them can be interpreted as waves of excitation and inhibition over time. In cells of inferior temporal cortex, analysis of the variance shows that fewer than 15 components are needed to completely represent the spike density functions, and the variation due to the stimulus patterns themselves could be represented with as few as three to five components. The other components probably represent noise carried with the signal.

The response of single units in area TE was markedly increased by some Walsh patterns, while it was completely inhibited by others. However, the response did not consist solely of a raising or lowering of the average firing rate; it was also temporally modulated. The principal components of the K-L transform were used to quantify the dependence of the temporal modulation on the stimulus pattern. Preliminary evidence indicates that, for each individual neuron, specific combinations of principal components correspond with certain Walsh patterns. Also, almost every cell encountered in area TE showed some consistent modulation of activity of one or more of the Walsh patterns. This suggests that the units in area TE are encoding features of visual stimuli in the temporal waveform of their activity. Further, since the K-L transform requires fewer components than any other linear transform (e.g. Fourier) for a given accuracy in representing the temporal waveform of activity, its complete

description cannot be made with a single measurement of the spike train. Nevertheless, our principal-component analysis does achieve a marked reduction in the dimensionality of the space that can be used to describe the encoding (from 64 individual Walsh patterns to 3-5 waveform components) by the individual neurons.

Having found that area TE neurons seem to carry information about 2-dimensional features in their temporal firing pattern, we felt that we must know whether this temporal encoding of visual information is limited to inferior temporal neurons or whether it occurs throughout the cortical visual system, including the striate cortex. We have therefore recorded single neurons in monkey striate cortex in order to determine what aspects of 2-dimensional pictures might be represented in the temporal activity of striate neurons. Although the receptive field properties of single neurons in area 17 have been studied extensively, receptive-field structure alone cannot predict a cell's temporal response to 2-dimensional patterns because such structure does not describe a spatial-to-temporal transformation.

Again we used the 64 black and white Walsh stimuli, though now reduced to 3 degrees square (a more appropriate size for receptive fields of striate neurons), and again the monkeys performed the blink task. The receptive fields of the neurons studied were approximately 3 degrees contralateral to the center of gaze.

Activity of these single units in striate cortex was found to represent members of the stimulus set differentially. Two to three components of the K-L transform were needed to reconstruct the cell's activity well enough to preserve its ability to differentiate among the stimuli. Again, since the K-L transform is the most efficient linear transform for representing the spike density functions, no single linear measurement can adequately represent the ability of these cells to discriminate among 2-dimensional patterns.

Some patterns which give the same response when measured with any single parameter, e.g. spike count, are differentiable when the waveform of the activity (as measured with multiple components) is considered. There was very little difference in the cell's ability to differentiate stimuli when the waveforms were analyzed over short (128ms) rather than long (384ms) periods. We conclude that single striate neurons, like area TE neurons, can convey detailed information about 2-dimensional visual features in the temporal modulation of their activity.

These results, which have now been obtained in both the first and last cortical visual stations, suggest that the waveform of temporal modulation in response to visual features is important for pattern processing throughout the visual system. Further, using the data collected from striate neurons, we have been able to reconstruct the spatial representations of the different temporal waveforms of the responses. These spatial representations of the first or tonic component of neuronal firing correspond closely to the responses evoked with the traditional slit of light, but the reconstructions often suggest greater spatial complexity than is revealed by the traditional

stimulus. Finally, the reconstructions based on the second or transient component often reveal a second spatial representation which is usually not suggested by traditional techniques.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Solving the problem of how the brain processes sensory information has been a major goal of psychologists and neurophysiologists. These experiments are an attempt to move closer to an understanding of such processing in the visual system of the primate. Since the inferior temporal region is involved in higher-order visual processing, we expect that our investigations will not only further our knowledge of how this cortex codes and stores visual stimuli but will also provide insight into visual perceptual and memory problems seen clinically as part of many disorders of the brain, both psychiatric and neurologic.

PROPOSED COURSE OF RESEARCH:

Experiments on the role of differing types of attention on the responses of single cells will be continued. Now that we have established that the temporal pattern of neuronal discharge carries information about spatial features, we will continue experiments to understand the encoding and its mechanism. Further, now that we are able to measure the information in a spike train quantitatively, we are in a position to determine how attentional factors alter the information content of the spike train. We are also in a position to determine how the information conveyed about complex images is built up throughout the visual system. For example, we can test the suggestion quantitatively that the pattern information encoded by inferior temporal neurons survives retinal translation or size change. In addition, an effort will be made to correlate single-cell results with behavioral results in normal and operated monkeys, as well as with psychophysical results we hope to gather in normal human subjects and patients with brain lesions. After the arrival of expected new equipment, we will begin experiments designed to determine whether the 2-dimensional pattern information in the spike train changes in inferior temporal neurons in relation to the particular perceptual or memory task required of the monkeys. Finally, if time and personnel permit, recording of single neurons in parts of the amygdala that receive the major inferior temporal output will be undertaken in an attempt to determine what transformation of the visual information has taken place at this first station of the limbic system.

PUBLICATIONS:

Richmond, B. J., Wurtz, R. H. and Sato, T. Visual response of inferior temporal neurons in the awake rhesus monkey. J. Neurophysiol (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER <div style="text-align: center;">Z01 MH 02033-06 LN</div>
PERIOD COVERED <div style="text-align: center;">October 1, 1982 to September 30, 1983</div>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <div style="text-align: center;">Functional mapping of sensory systems</div>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <div style="text-align: center;">Kathleen A. Macko, Staff Fellow, LN, NIMH</div>		
COOPERATING UNITS (if any) <div style="text-align: center;">Laboratory of Cerebral Metabolism, NIMH Laboratory of Psychology and Psychopathology, NIMH</div>		
LAB/BRANCH <div style="text-align: center;">Laboratory of Neuropsychology</div>		
SECTION		
INSTITUTE AND LOCATION <div style="text-align: center;">NIMH, NIH, Bethesda, MD 20205</div>		
TOTAL MANYEARS: <div style="text-align: center;">0.5</div>	PROFESSIONAL: <div style="text-align: center;">0.5</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="text-align: justify;"> <p>The [¹⁴C] 2-deoxyglucose method was used to identify the cerebral areas related to vision through a comparison of glucose utilization in a visually stimulated as compared to a visually deafferented hemisphere in the <u>rhesus monkey</u>. Cortically, the visually related areas included the entire expanse of striate, prestriate, and <u>inferior temporal cortex</u> as far forward as the temporal pole, the posterior part of the <u>inferior parietal lobule</u>, and the <u>prearcuate</u> and <u>inferior prefrontal cortex</u>; subcortically, in addition to the lateral geniculate nucleus and superior colliculus, visually related structures included large parts of the <u>pulvinar</u>, <u>caudate</u>, <u>putamen</u>, <u>claustrum</u>, and <u>amygdala</u>. These results, which are consonant with a model that postulates an occipito-temporo-prefrontal pathway for <u>object vision</u> and an occipito-parieto-prefrontal pathway for <u>spatial vision</u>, reveal the full extent of those pathways and localize their points of contact with limbic, striatal, and diencephalic structures.</p> </div>		

Principal Investigator:

K.A. Macko Staff Fellow

LN NIMH

Other Investigators:

M. Mishkin	Chief, Laboratory of Neuropsychology	LN NIMH
J. Bachevalier	Guest Worker	LN NIMH
C. Kennedy	Guest Worker	LCM NIMH
L. Sokoloff	Chief, Laboratory of Cerebral Metabolism	LCM NIMH
R.K. Nakamura	Senior Staff Fellow	LPP NIMH

PROJECT DESCRIPTION:

Converging evidence from studies of lesion effects, anatomy, and single units indicates that, in the primate, the system for processing information about visual objects extends beyond the striate cortex to include the circumstriate and inferior temporal areas. From the inferior temporal area, in turn, information about these objects appears to be transmitted to subcortical structures in the temporal lobe and to inferior prefrontal cortex. The same classical mapping techniques have also been used to identify another system, specialized for processing information about spatial vision, which also depends on a corticocortical pathway, in this case from striate through prestriate to inferior parietal and dorsal prefrontal cortex. In addition, both systems are known to have subcortical inputs to, and projections from, structures in the tectofugal pathway. Despite a range of research efforts, however, the full extent and precise boundaries of these cortical pathways are still undefined, as are their exact points of contact with their subcortical forebrain targets. The [^{14}C] 2-deoxyglucose method for measuring local cerebral glucose utilization (LCGU) has provided a means of clarifying these issues.

Experiment 1:

To map the functional visual system by means of the 2-deoxyglucose technique, we prepared monkeys with a unilateral optic tract section alone or a tract section combined with section of the forebrain commissures, thus visually deafferenting one hemisphere while leaving the other intact. This made it possible to compare LCGU values in a "seeing" and a "blind" hemisphere within the same animal and thereby map the visually related areas.

Monkeys in this initial study sat in a primate chair while visual patterns mounted on a drum rotated around their heads. They were injected with a pulse of radioactively labeled 2-deoxyglucose and monitored over the next 45 minutes to determine the levels of free deoxyglucose in the blood. Most of the radioactively labeled substance is incorporated into active cells during this period, and any extracellular deoxyglucose is of sufficiently low concentration that its presence does not contaminate the autoradiographs.

In a second study, monkeys prepared surgically as above were trained on a visual pattern discrimination task in which they were required to respond with the hand opposite the blind hemisphere. In this task a positive stimulus was paired with one of a series of negative stimuli in sequential blocks of trials, and correct responses were reinforced with a water reward.

In both behavioral situations, reduced glucose utilization in the blind as compared with the seeing hemisphere was seen cortically not only in the geniculostriate system, but throughout the entire expanse of circumstriate and inferior temporal cortex and reaching even to the inferior prefrontal cortex. The functionally depressed zone included tissue adjacent to the inferior temporal cortex in the upper bank of the superior temporal sulcus and in the fusiform and perirhinal areas. Subcortically in the temporal lobe, side-to-side differences were seen in lateral and dorsal amygdala, ventral putamen, ventral claustrum, and tail of caudate. Outside this stimulus-processing system specialized for object vision, asymmetries were also seen in the inferior parietal lobule and prearcuate region of the frontal lobes, the tissue specialized for spatial vision. Performance on the discrimination task led to an asymmetrical increase of glucose utilization in structures associated with the active hand and to a symmetrical increase in structures associated with the act of drinking. In the cortical tissue related to vision the effects of the two different behavioral situations were substantially the same. In the subcortical tissue related to vision, however, the monkeys performing the visual discrimination task showed reduced left-right hemispheric asymmetries compared to those of the passively stimulated group in parts of the putamen and caudate nucleus. Such changes occurred within the body and head of the caudate, the lateral and basal amygdala, and the medial pulvinar. In these structures, left-right asymmetries virtually disappeared, due mainly to dramatic increases in right hemisphere activity presumably related to asymmetrical input from the somatosensory system. The body and head of caudate, parts of the amygdala, and medial pulvinar thus appear to serve multimodal functions in that visual activation of these loci in the left hemisphere was balanced by somatosensory activation of the same loci on the right.

Computer-enhanced images of the autoradiographic brain sections from the animals in this experiment were examined in detail in order to delineate the exact borders of visually related tissue in the parietal and temporal lobes. We found that the cortical visual/nonvisual borders 1) were sharp and highly consistent among the animals, 2) outlined more visual tissue than expected in both the parietal and temporal lobes, and 3) appeared reliably at zones of architectonic transition.

Our data show that behind the junction of the lunate and the intraparietal sulcus all cortical tissue is related to vision. In front of this junction, nonvisual tissue first appears at the anterior or upper lip of the intraparietal sulcus. In the cross sections, one visual/nonvisual border can be placed on the medial surface, and one on the upper bank of the intraparietal sulcus. Anteriorly, both borders move in a ventral direction, with all tissue below them remaining visual. Still more anteriorly, this

single expanse of visually related tissue is separated into two parts, parietal and temporal, by the appearance of nonvisual tissue in the lateral fissure and on the superior temporal gyrus.

In the parietal lobe, the upper border is always within the intraparietal sulcus, about halfway down the upper bank caudally and closer to the fundus rostrally. The lower border moves out of the lateral fissure and remains on the cortical surface close to the upper lip of the lateral fissure, and then it moves into the intraparietal sulcus rostrally. The rostral limit of visual tissue is within the intraparietal sulcus, about 5mm behind its anterior tip.

In the temporal lobe the upper border is always within the superior temporal sulcus, generally about halfway down the anterior (or dorsal) bank of the superior temporal sulcus but within the fundus rostrally. Anteriorly, the lower border moves from the calcarine fissure to the hippocampal sulcus (where it continues midway along its length) and then turns laterally to enter the occipitotemporal sulcus and finally the fundus of the rhinal sulcus.

These visual/nonvisual borders generally appear at zones of cytoarchitectonic transition described by von Bonin and Bailey. For example, in the parietal lobe a visual/nonvisual border appears on the lateral surface near the zone of transition between areas PG and PF and on the medial surface between prestriate area OA and parietal area PE. Also, in the temporal lobe, visual/nonvisual borders appear in the transition zones between TF and TH, TE and TH, and TE and TG. Finally, inside the expanse of visually related cortex, metabolic borders appeared to separate architecturally different subareas, as in the lower bank of the intraparietal sulcus and in the upper bank of the superior temporal sulcus. These results lend new functional validity to cortical architectonics.

Experiment 2:

Portions of each of the visual areas within the cortical pathway serving object vision are known to be reciprocally connected through the forebrain commissures. In particular, the representation of the vertical meridian at the OC-OB border as well as selected parts of area OA receive commissural inputs via the splenium of the corpus callosum, while extensive portions of areas TE0 and TE receive contralateral input via both the splenium and the anterior commissure. Since the transfer of visual information between the hemispheres is critically dependent on these reciprocal connections, we attempted to localize and to quantify the contribution to vision made by the commissural systems. To do this we measured LCGU throughout the cortical visual system in two different surgical preparations: unilateral optic tract section combined with forebrain commissurotomy, and unilateral optic tract section alone. The 2-deoxyglucose method was applied one month postoperatively in awake rhesus monkeys actively viewing visual patterns. The commissural contributions to vision were inferred from differences in LCGU between the deprived hemispheres of the two groups.

From the autoradiographs of each brain, representative sections were chosen at 1mm intervals throughout the extent of the cortical visual pathway. Weighted averages of LCGU for the entire extent of each visual area were then calculated from these sections by means of a computerized image-processing system. In the intact hemisphere there was a progressive decline in LCGU along the cortical visual pathway from a high of 66 umoles/100g/min in area OC to a low of 47 in anterior TE. This sequential decline in the intact hemisphere was the same both for the animals with tract section plus commissurotomy and for those with tract section only. There were also no differences between operated groups in the visually deprived hemisphere for areas OC through TEO, where LCGU averaged 50% of that in the intact hemisphere (ranging from 40% in OC to 60% in TEO). A difference attributable to visual input via the intact commissures was found in TE, however, where LCGU in animals with combined tract section and commissurotomy remained at 60% of that in the intact hemisphere, whereas in animals with tract section only LCGU reached 80% and 90% of the values in the intact hemisphere for posterior and anterior TE, respectively. These results indicate that commissural inputs contribute more to the visual functions of area TE than to those of any other visual area and that, in fact, commissural inputs alone may be insufficient to support visual function in areas TEO, OA, and the OC-OB border.

Experiment 3:

The results of Experiment 2 have presented us with a paradox. The rise in metabolic activity in the deprived hemisphere of the tract-cut preparations clearly reflect the functional contribution of the forebrain commissures in the anterior portion of the occipito-temporal pathway but, surprisingly, not in the posterior portion. Thus, on the one hand, our metabolic data on area TE are in good accord with existing anatomical data, which indicates that area TE receives interhemispheric projections through both the splenium and the anterior commissure. On the other hand, substantial commissural fiber projections also reach the more posterior visual areas - specifically, the OC-OB border, OA, and TEO. Yet our metabolic data give no indication of this heavy and widespread projection. A possible explanation of this paradox is that, unlike the commissural input to area TE, which clearly can support visual function by itself, the commissural input to the more posterior zone may be effective only against a background of spontaneous activity provided by an intact but visually occluded retino-geniculo-cortical pathway.

In order to test this hypothesis, we have prepared a series of monkeys in which a "blind" right hemisphere was produced by midline section of the optic chiasm combined with occlusion of the right eye rather than by right optic tract section. Through a comparison of LCGU in the right hemispheres of these animals and of those studied previously with right optic-tract section, the functional effectiveness of commissural input with and without spontaneous retinal input can be evaluated. Greater metabolic activity in the former case would indicate that the commissural fibers to the prestriate-posterior temporal zone do require a minimum level of background activity from the intact retina in order to make a functional contribution to vision.

Preliminary qualitative analysis, however, shows no reliable difference between animals with a tract cut and those with a chiasm cut and occluded eye either at the OC-OB border or within areas OB, OA, or TEO. In short, the commissural contribution to vision in the prestriate-posterior temporal zone appears not to be augmented by the presence of intact retinal fibers and spontaneous retinal activity. The functional difference between the commissural fibers serving the anterior and posterior portions of the occipito-temporal visual pathway thus requires some other explanation. A possible solution has been suggested in another project from this laboratory (see Project MH 02036, Neural coding of visual stimuli in the immobilized monkey).

Experiment 4:

In order to trace the functional development of the visual system, we are conducting studies in infant monkeys similar to those described in the first experiment. Thus far, a series of animals were prepared with unilateral optic-tract section combined with forebrain commissurotomy at 1 day, 1 week, and 1, 2, 3, and 5 months of age. The 2-deoxyglucose method was then applied an average of two days postoperatively in the two younger animals and one month postoperatively in the others. As with the adults, representative brain sections were chosen at 1mm intervals throughout the entire cortical visual pathway in each animal, and weighted averages of LCGU for the entire extent of each visual area were calculated. The results show that there are systematic age-related changes both in the absolute level of LCGU within the normal seeing hemisphere and in LCGU differences between the normal left and the deprived right hemispheres.

In all cortical visual areas of the intact hemisphere, LCGU was lowest in the youngest subjects, peaked at 4 months, and then declined in the 6-month-old subject to levels found in adults. As in adults, the intact hemisphere of infants shows a progressive decline in LCGU along the ventral cortical visual pathway from a high in area OC (ranging from 26.1 umoles/100g/min at 9 days to 88.1 at 4 months) to a low in anterior TE (ranging from 17.6 at 2 days to 59.7 at 4 months). This gradient was present in all subjects, but was most shallow in the two youngest.

The deprived hemisphere showed reduced LCGU relative to the normal hemisphere in all areas of the cortical visual pathway at all ages. Also at all ages, hemispheric differences were greatest in area OC and smallest in anterior TE. These differences, however, varied systematically with the age of the animal. Thus, for each cortical area, the relative difference between the normal and deprived hemispheres was smallest in the youngest subjects and approached the differences seen in adults only at about four months of age, the time at which LCGU appeared to peak.

This finding that adult levels of metabolic activity are not reached until about 4 months of age is consistent with behavioral data (see Project MH 02038, Ontogenetic development of memory and habit formation) indicating that

the neural capacity for visual object recognition is probably not developed until about this time.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Since energy metabolism and functional activity are highly correlated within the nervous system as demonstrated by these and other studies, the 2-deoxyglucose method can provide a deeper understanding than has been possible before of the role of various cerebral structures in behavior through both the identification of the structures involved in a particular behavior and the quantification of the degree of their participation. Since it is only through a knowledge of what is normal that we gain insight into what is abnormal, such understanding is certain to contribute greatly to the ultimate goal of diagnosing and treating a wide variety of neurological and mental disorders.

PROPOSED COURSE OF RESEARCH:

Our goal is to apply the 2-deoxyglucose method to the study of a variety of behavioral processes in the monkey, including perception, attention, memory, emotion, and volition for the purpose of identifying the various structures involved in these different behaviors and quantifying the degree of their participation. Our immediate plans are to investigate the neural structures involved in visual memory, specifically object recognition, a process which we believe involves not only the visual system but also parts of the limbic system (amygdala and hippocampus) and the medial thalamus.

In these experiments 2-deoxyglucose will be administered to monkeys performing a running recognition task designed to "load" the visual memory system throughout the experimental session. As in our previous studies, the monkeys will be prepared with a right optic tract section and section of the forebrain commissures to permit the flow of visual information through the left hemisphere only. By taxing visual memory we hope to see increased LCGU in parts of the limbo-thalamic memory system (e.g., hippocampus and medial thalamic nuclei) in which they were not seen before.

We also plan to continue our investigation of the development of the visual system in infant monkeys, first completing the normative study under conditions of passive visual stimulation and then attempting to parcel out developmental differences between the "habit" and the "memory" systems (see Project MH 00478, Neural mechanisms of memory and habit formation).

PUBLICATIONS:

Macko, K.A., Jarvis, C.D., Kennedy, C., Miyaoka, M., Shinohara, M., Sokoloff, L., and Mishkin, M.: Mapping the primate visual system with 2-[¹⁴C]deoxyglucose. Science, 218: 394-397, 1983.

Mishkin, M., Ungerleider, L.G., and Macko, K.A.: Object vision and spatial vision: Two cortical pathways. Trends in Neuroscience (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02034-04 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Subcortical mechanisms related to frontal lobe functions in the monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) H. Enger Rosvold, Research Psychologist, LN, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Project terminated due to retirement of the Principal Investigator June 30, 1982.</p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02035-03 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Anatomy of the primate visual system		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Leslie G. Ungerleider, Senior Staff Fellow, LN, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 1.0	OTHER: 3.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The <u>sensory processing</u> , <u>perception</u> , and <u>memory</u> of visual events require the transmission of neural activity across a corticocortical, multisynaptic pathway from <u>striate cortex</u> , or <u>area 17</u> , through several <u>prestriate</u> "association areas". In addition, visual input from striate cortex reaches these prestriate areas indirectly via two subcortical visual structures, the <u>superior colliculus</u> and the <u>pulvinar</u> . By the combined use of <u>neuroanatomical techniques</u> , <u>electrophysiological recording</u> , and newly developed <u>histological staining</u> procedures, we have been identifying the multiple prestriate visual areas in the <u>macaque</u> , mapping their interconnections, and tracing the flow of visual information forward to the still higher-order visual areas located within the <u>temporal</u> and <u>parietal</u> lobes, visual areas that are critical for <u>object vision</u> and <u>spatial vision</u> , respectively.		
(545)		

Principal Investigator:

L.G. Ungerleider Senior Staff Fellow

LN NIMH

Other Investigators:

M. Mishkin Chief, Laboratory of Neuropsychology

LN NIMH

R. Desimone Senior Staff Fellow

LN NIMH

Technical Support Staff:

T.W. Galkin Psychologist

LN NIMH

F.D. Lewis Stay-in-School Student

LN NIMH

J.N. Sewell III Biological Laboratory Technician (Animal)

LN NIMH

PROJECT DESCRIPTION:

Striate cortex, the primary visual cortex, is the source of two major corticocortical, multisynaptic visual pathways. One of these follows the course of the superior longitudinal fasciculus, interconnects the striate, prestriate, and posterior parietal areas, and appears to be important for spatial vision. The other follows the course of the inferior longitudinal fasciculus, interconnects the striate, prestriate, and inferior temporal areas, and is critical instead for object vision. Although visual information must reach the posterior parietal and inferior temporal cortex to enable their participation in spatial vision and object vision, respectively, the complex circuitry through which this information is transmitted has yet to be unraveled. We have undertaken to examine the details of the connections within these two cortical visual pathways, beginning with an analysis of the projections of the striate cortex itself.

Experiment 1:

Anatomical material from two series of monkeys (*Macaca mulatta*) was used to determine the locus, extent, and visuotopic organization of striate projections to prestriate cortex. One series was processed for terminal degeneration by the Fink-Heimer procedure following unilateral lesions of lateral, posterior, or medial striate cortex, areas representing central, peripheral, and far peripheral vision, respectively. Collectively, the lesions included all of area 17 with little or no invasion of area 18. The second series was processed for autoradiography following tritiated amino-acid injections into various striate sites representing the center of gaze and eccentricities ranging from 0.5° to 45° in either the upper or lower hemifield. The results indicate that striate cortex (cytoarchitectonic area OC), or V1, projects to at least three separate and topographically organized

visual areas within prestriate cortex: V2, a circumstriate cortical belt; MT, located on the posterior bank and floor of the superior temporal sulcus; and V3a, located at the fundus of the posterior intraparietal sulcus. V2 corresponds to area OB, while MT and V3A are both contained within area OA.

Now that the total system of striate-prestriate projections has been delineated, the effects of completely disconnecting inferior temporal from striate cortex can finally be investigated. Accordingly, monkeys are being prepared with complete lesions of just the three striate projection fields described above (without inclusion of any striate tissue) prior to testing them on visual pattern discriminations. In addition, we are trying to determine whether even small prestriate lesions might disconnect inferior temporal cortex from corresponding parts of the visual field by testing discrimination of stimuli confined to those parts of the field in monkeys that have been trained to maintain fixation. In view of the clear visuotopic organization of the striate projection zones in both V2 and MT, even limited lesions within these prestriate zones should yield severe visual deficits provided the animals are forced to use the part of the visual field corresponding to the area damaged. To monitor fixation accurately, we are making use of a magnetic eye-coil system.

Experiment 2:

Having delineated the projections of striate to prestriate cortex, which we found to consist of three separate re-representations of the visual field, we are now following these projections further, with the inferior temporal and posterior parietal cortex as our targets. Since the multiple prestriate areas can be identified not only by their approximate location, but also by their distinct electrophysiological properties, all autoradiographic studies are now being performed under physiological control. By recording the activity of neurons from the microsyringe needle and mapping receptive fields, we are able to inject tritiated amino acids into portions of each prestriate area that represent known parts of the visual field. To date, this recording technique has been used for injections into both V2 and MT. In addition to tracing the projections of these two areas, we have developed a myeloarchitectural stain to distinguish among them and other prestriate regions.

Our results indicate that V2 projects to two visual areas located anterior to it, V3 and V4. Together, these three prestriate areas are arranged in adjacent belts that nearly surround the striate cortex, and, like striate cortex, each belt contains a representation of the visual field, with the upper field located ventrally and the lower field, dorsally. Other studies have shown that V4, in turn, projects to both areas TEO and TE in the inferior temporal cortex. In contrast to V2, which appears to provide a major link forward from striate cortex into the temporal lobe, our results on MT suggest that it provides a major link from striate cortex into the parietal lobe via its projections to four additional areas in the superior temporal and intraparietal sulci. (These projection areas of MT, unlike those of V2, are highly convergent, with only a suggestion of topography.) It thus appears that there is a divergence in the flow of visual information from striate

cortex which begins at the first prestriate areas. Whereas information from V2 is mainly directed ventrally into the temporal lobe, information from MT is mainly directed dorsally into the parietal lobe, these two divergent projection systems providing the anatomical substrate for object vision and spatial vision, respectively.

Experiment 3:

Anatomical material prepared for Experiment 1 was used to investigate subcortical efferents from striate cortex to the pulvinar, a nucleus in the thalamus implicated in attentional mechanisms. We had found in Experiment 1 that striate projections to the prestriate cortical area V2 are visuotopically organized, and we had prior evidence of a similar topographic arrangement of pulvinar projections to V2. In the present study, we found that there is also a precise visuotopic organization of striate projections to the pulvinar, indicating the existence of two sources of striate input to V2 that are in perfect register: one, direct, i.e., corticocortical; and the other, indirect, via the pulvinar. This parallel system of inputs to V2 thus provides a possible mechanism by which activating signals (e.g., from dorsal cortical areas to the pulvinar via midbrain structures) acquire visual field specification, that is, a mechanism that tells the organism where in the field to attend. Although this hypothetical circuit may apply to all sensory systems, for the visual modality our data indicate that only the inferior and lateral nuclei of the pulvinar are involved. Future anatomical studies will investigate the sources of midbrain input to the inferior and lateral pulvinar, as well as the organization of afferents to the pulvinar from other sensory modalities. In addition to the anterograde tracing techniques of degeneration and autoradiography, these studies will employ horseradish peroxidase for tracing retrograde axonal transport.

Experiment 4:

Anatomical material from Experiment 2 was used to investigate the location and topographic organization of the subcortical projections from areas V2 and MT. Our results indicate that both V2 and MT, like striate cortex, project topographically to the inferior and lateral pulvinar, the superior colliculus, and the reticular nucleus of the thalamus. MT projects, in addition, to the putamen, caudate, claustrum, and pontine grey, subcortical structures to which V4 has also been shown to project. Thus, the subcortical projections of MT and V4 are more extensive than those of either striate cortex or V2. The considerable overlap in the subcortical projections from MT and V4 suggests that the contribution of each area to subcortical processing lies not in a unique set of subcortical projections but rather in the unique information each supplies, namely, direction of stimulus motion for MT and stimulus form and color for V4 (see Project MH 02036, Neural coding of visual stimuli in the immobilized monkey).

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

An understanding of the basic mechanisms mediating normal vision is the first step in the prevention, diagnosis, and alleviation of sensory, perceptual, and mnemonic disorders. To this end, we have been exploring the projections of striate cortex, both to prestriate "association areas" and to subcortical visual structures. Our goal is to unravel the complex system of projections to the still higher-order visual areas located within the parietal and temporal cortex, areas critical for spatial vision and object vision, respectively. The combined use of axonal transport techniques and electrophysiological recording provides a powerful tool for tracing neural connections within these central visual pathways. In addition, the recent development of highly selective histological stains may give us the opportunity for the first time of identifying higher-order visual areas in the human brain that we have identified in the monkey.

PROPOSED COURSE OF RESEARCH:

To understand the role of visual association cortex in perception and memory we must identify the multiple functional areas that comprise this cortex, delineate their topographic organization, and explore the complex circuitry of their interconnections. So far, we have discovered that striate cortex is the source of two divergent cortical pathways, each with its own set of hierarchically organized prestriate association areas. We plan to study the further projections of these two pathways stepwise to the still higher-order visual areas located within the temporal and parietal lobes. A major question for the future will be how the object and spatial information carried in these two separate pathways are subsequently integrated anatomically to yield a unified visual percept. Ultimately, we will explore the links of both pathways to affective, memory, and motor systems by examining the projections of the multiple visual association areas to the limbic system, the prefrontal cortex, and the striatum.

PUBLICATIONS:

Mishkin, M., Lewis, M.E., and Ungerleider, L.G.: Equivalence of parieto-preoccipital subareas for visuospatial ability in monkeys. Behav. Brain Research 6:41-55, 1982.

Mishkin, M. and Ungerleider, L.G.: Contributions of striate inputs to the visuospatial functions of parieto-preoccipital cortex in monkeys. Behav. Brain Research 6:57-77, 1982.

Ungerleider, L. G., Galkin, T. W., and Mishkin, M.: Visuotopic organization of projections from striate cortex to inferior and lateral pulvinar in rhesus monkey. J. Comp. Neurol. 217: 137-157, 1983.

Ungerleider, L.G., Desimone, R., Galkin, T.W., and Mishkin, M.: Subcortical projections of area MT in the macaque. J. Comp. Neurol. (in press), 1983.

Ungerleider, L. G.: Contrasts between the corticocortical pathways for pattern and spatial vision. In C. Chagas (Ed.) Study Group on: Pattern Recognition Mechanisms, The Pontifical Academy of Sciences, Vatican City (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02036-03 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neural coding of visual stimuli in the immobilized monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Robert Desimone, Senior Staff Fellow, LN, NIMH		
COOPERATING UNITS (if any) Section on Visual Processing, CB, NEI		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The neural mechanisms for the <u>visual recognition</u> of objects extend beyond striate cortex into the surrounding prestriate and inferior temporal areas. Neurons in the prestriate areas appear to process local properties of objects such as the location of boundaries, while inferior temporal neurons appear to process more global features such as object shape. We are studying <u>single neurons</u> in prestriate and inferior temporal cortex to investigate both mechanisms. In <u>prestriate cortex</u> we have found that one area, <u>area MT</u>, is specialized for analyzing <u>stimulus motion</u> and contains <u>direction-of-motion columns</u> similar to the orientation columns discovered in primary visual cortex. In another area, <u>area V4</u>, we have found that neurons are sensitive to the <u>length</u>, <u>width</u>, and <u>color</u> of object contours and may play a role in separating figure from ground. In <u>inferior temporal cortex</u> we found that over half the neurons were tuned to a set of <u>shape descriptors</u> that can be used to code object shape. Since different neurons are tuned to different descriptors, a population of inferior temporal neurons could code any shape. </p>		
(551)		

Principal Investigator:

R. Desimone	Senior Staff Fellow	LN NIMH
-------------	---------------------	---------

Other Investigators:

E.L. Schwartz	Assistant Professor	NYU Med. School
C.G. Gross	Professor	Princeton Univ.
M. Mishkin	Chief, Laboratory of Neuropsychology	LN NIMH
S.J. Schein	Expert	CB NEI
F.M. DeMonasterio	Chief, Section Visual Processing	CB NEI

Technical Support Staff:

J.C. Moran	Psychology Technician	LN NIMH
------------	-----------------------	---------

PROJECT DESCRIPTION:

Previous work in this and other laboratories has shown that the neural mechanisms for the recognition of objects extend beyond the primary visual cortex into the surrounding prestriate and inferior temporal visual areas. Neurons in the prestriate areas receive their visual input directly or indirectly from striate cortex, have small receptive fields, and appear to process the local features of objects, such as individual contours and surfaces. Neurons in inferior temporal cortex receive their visual input from prestriate cortex, have large receptive fields, and appear to process the global features of objects, such as their shape. In this project we are studying both types of processing, local and global, in prestriate and inferior temporal cortex. To study the passive visual properties of neurons, unaffected by eye movements or the changing state of the animal, we are recording neural activity primarily in the immobilized, lightly anesthetized macaque. We thus have complete control over the pattern of stimulation on the retina, and we are able to study individual neurons in this way for many hours. In addition, we have recently begun supplementing the unit recordings in immobilized monkeys with both unit recordings and local metabolic mapping in awake behaving monkeys.

Experiment 1: Direction-of-motion columns in prestriate cortex:

The concept of columnar organization, i.e., the subdivision of the major structures of the brain into columns or modules of neurons united by a common task, is of fundamental importance in understanding the organization of the nervous system. In the visual system the only columnar systems discovered so far have been in the primary visual cortex. As described by Hubel and Wiesel, the primary visual cortex contains elaborate columnar systems for analyzing stimulus orientation and ocular dominance. If we could find columnar organization for other stimulus dimensions within the prestriate visual areas,

this would provide us with the best evidence yet of the functions of these areas. We chose the prestriate area 'MT' to look for such an organization because the location, borders, topographic organization, and anatomical connections of MT have already been well established by other experiments in the laboratory.

We discovered that area MT contains a columnar architecture for analyzing the direction of stimulus motion. In three monkeys, we recorded from 614 single neurons on 21 electrode penetrations. The majority of cells respond to a single direction of stimulus motion within their receptive field. In a vertical column of cells, all cells have the same receptive field and respond to the same direction of motion. Moving horizontally within the cortex, the optimal direction of motion changes smoothly and systematically from column to column. The representation of direction of motion in MT is strikingly similar to the representation of orientation in striate cortex. Even the size of the columnar systems is similar - 180 degrees of direction of motion in MT is represented within a piece of cortex 400 to 500 microns wide, the same size as the representation of 180 degrees of stimulus orientation in striate cortex. These results suggest that just as the analysis of stimulus orientation is a fundamental function of striate cortex, the analysis of stimulus direction of motion is a fundamental function of area MT. Anatomical experiments in the laboratory indicate that MT sends this information primarily to the spatial system of the parietal lobe but also, through intermediate projections, to the pattern system of the temporal lobe.

Experiment 2: Analysis of form and color in prestriate cortex:

While MT neurons are sensitive to stimulus motion, we have found neurons in another prestriate area, area V4, that are sensitive to the form and color of stationary stimuli. Although our experiments are still in progress, it is already clear that V4 neurons are sensitive to many different local features of objects, including length, width, orientation, color, contrast, and spatial frequency. Unlike neurons in striate cortex, the receptive fields of V4 neurons are surrounded by large, silent suppressive regions with specific form and color properties. In both anesthetized, immobilized monkeys and in awake monkeys trained to fixate, we have found that stimuli placed outside of the receptive field are without effect themselves, yet are able to completely suppress the response to a similar receptive-field stimulus. Thus, many V4 neurons respond to a stimulus only if it stands out from its background on the basis of a difference in color or form. These neurons may thus play a role in separating 'figure' from 'ground', a fundamental task in visual perception. Anatomical experiments in the laboratory indicate that V4 sends this form and color information to the object recognition system of the temporal lobe.

Like other prestriate areas, V4 contains a representation of the contralateral visual field. Within the central visual field, V4 receptive fields rarely extend more than 10° across the vertical meridian into the ipsilateral visual field. Yet, V4 receives heavy commissural projections from the opposite hemisphere not limited to the representation of the vertical meridian. What

is the purpose of these projections? To test whether the commissural projections might contribute to the large suppressive surrounds of V4 receptive fields, we measured the extent of the suppressive surrounds of individual V4 neurons within the ipsilateral hemifield. We found that even though the excitatory receptive fields of V4 neurons were confined to the contralateral hemifield, the suppressive surrounds extended up to 16° across the vertical meridian into the ipsilateral visual field. The ipsilateral suppression was significantly reduced following section of the corpus callosum. The commissural inputs to V4 thus appear to be largely suppressive and may serve to integrate the figure/ground mechanisms in the two hemispheres.

Experiment 3: Shape recognition and inferior temporal cortex:

A likely site for mechanisms of shape recognition is inferior temporal (IT) cortex. In man and monkey, removal of this area impairs visual recognition of shapes and patterns while leaving basic visuosensory capabilities intact. Furthermore, unlike neurons in prestriate cortex, many IT neurons are sensitive to the overall shape of objects rather than the location and quality of individual edges and contours.

In this study we examined how IT cortex might extract information about the overall shape of an object from information about its boundary. We adopted a method of representing shapes in terms of local boundary orientation that is used in computer pattern-recognition systems. The method depends on extracting a set of periodic features, known as the Fourier Descriptors, from the boundary of the object. Any shape is fully described by its set of Fourier Descriptors, or FDs, and a smaller set of only the low-frequency terms can often provide the 'gestalt' of a shape. Furthermore, this method of describing shape is independent of both the position and size of the stimulus. Thus, the FDs are a powerful and efficient alphabet for representing and classifying shapes.

Could IT neurons code shape on the basis of global shape features like the Fourier Descriptors? To explore this possibility, we created a set of stimuli from single FDs. If IT neurons function as 'bandpass filters' for shape, one would expect different IT neurons to be tuned to different FD stimuli, and the tuning should be relatively independent of the size and position of the shape on the retina. The activity of a set of such neurons could specify or code any complex shape.

We studied 234 neurons in five monkeys. About half of the neurons were tuned to different FD stimuli. For two-thirds of the tuned cells, the shape of the tuning curve remained invariant over changes in the size of the stimulus and in its position on the retina. These results support the possibility that the visual system, and inferior temporal cortex in particular, use periodic shape descriptors in classifying objects. We now plan to test whether the responses of neurons to FD stimuli can be used to predict their response to complex objects.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

The primate, including man, is a highly visual animal. Thus, it is not surprising that perhaps half of the primate cerebral cortex is devoted directly or indirectly to visual processing. Consequently, the study of neural mechanisms of vision is not only of fundamental importance for our understanding of visual perception and memory but also for our understanding of brain function in general. The extrastriate visual areas described in this project are of particular importance to the field of neurobehavioral research because they contain the neural mechanisms for visual memory, and they are the direct source of nearly all the visual information to the limbic affective and motivational systems.

PROPOSED COURSE OF RESEARCH:

Our findings in the past year that neurons in one prestriate area are organized within direction-of-motion columns, that neurons in a different prestriate area code contour and color, and that neurons in inferior temporal cortex appear to code object shape all indicate that the study of single neurons can give us valuable insight into the neural mechanisms of perception and memory. Clearly we have only scratched the surface. We hope to follow the flow of information about motion into the parietal visuospatial system and study how parietal neurons use that information to code the spatial relations among objects. In addition, within the system for pattern vision, we plan to study how prestriate neurons use information about contours and colors to separate figure from ground and how inferior temporal neurons integrate information about object shape with other object features, such as texture, depth, and color. Finally, we will study the spatial distribution of these properties throughout the extrastriate cortex using metabolic mapping techniques.

PUBLICATIONS:

Albright, T.D., Desimone, R., and Gross, C.G.: Columnar organization of directionally selective cells in visual area MT of the macaque. J. Neurophysiol. (in press), 1983.

Schwartz, E.L., Desimone, R., Albright, T.D., and Gross, C.G.: Shape recognition and inferior temporal neurons. Proc. Nat. Acad. Sci. (in press), 1983.

Gross, C.G., Desimone, R., and Albright, T.D., and Schwartz, E.L.: Inferior temporal cortex as a visual integration area. IBRO Monograph Series (in press), 1983.

Gross, C.G., Desimone, R., Albright, T.D., and Schwartz, E.L.: Inferior temporal cortex and pattern recognition. In C. Chagas (ed.): Working Group on Pattern Recognition Mechanisms, Pontifical Academy of Sciences, Vatican City (in press), 1983.

Desimone, R., Schein, S.J., and Albright, T.D.: Neural mechanisms for form, color and motion analysis in prestriate cortex of the macaque. In C. Chagas (ed.): Working Group on Pattern Recognition Mechanism, Pontifical Academy of Sciences, Vatican City (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02037-02 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional anatomy of the somatosensory cortex of the monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David P. Friedman, Senior Staff Fellow, LN, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH Section on Brain Biochemistry, NSB, NIMH Northwestern University		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1.0	OTHER: 1.5
CHECK APPROPRIATE BOXES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The pathway by which somatosensory information reaches the limbic structures in the temporal lobe known to be critical for tactile memory has not yet been identified. To trace this pathway, the anatomical connections of identified <u>somatosensory fields</u> lying in or near the lateral sulcus of the <u>macaque monkey</u> have been investigated using both <u>anterograde</u> and <u>retrograde axonal transport techniques</u>. The data show that a series of <u>parallel tactile processing pathways</u> converge on the <u>insular cortex</u>; this region, in turn, projects directly to the amygdala and indirectly to the hippocampus via the rhinal cortex, thus linking the <u>somatosensory cortices</u> with the <u>limbic structures</u> of the temporal lobe.</p> <p>Opiates have been shown to affect <u>learning and memory</u>, though the locus of this action is not yet known. Combined <u>lesion and receptor binding studies</u> suggest that there are <u>opiateergic projections</u> from the amygdala to higher order cortical sensory processing areas, like the anterior insula and orbitofrontal areas. Furthermore, metabolic studies link the level of <u>mu receptors</u> to the rate of <u>protein phosphorylation</u> in the F₁ band, suggesting that opiates may help control <u>learning-related protein phosphorylation</u>.</p>		
(557)		

Principal Investigator:

D.P. Friedman Senior Staff Fellow

LN NIMH

Other Investigators:

E.A. Murray Staff Fellow

LN NIMH

M. Mishkin Chief, Laboratory of Neuropsychology

LN NIMH

R.J. Schneider Guest Worker

LN NIMH

C.B. Pert Chief, Section on Brain Biochemistry

NSB NIMH

A. Pert Research Psychologist

BPB NIMH

A. Routtenberg Professor

Northwestern Univ.

Technical Support Staff:

J.B. O'Neill Biologist

LN NIMH

PROJECT DESCRIPTION:

Objectives:

Work in this laboratory has shown that the amygdala and hippocampus are critical not only for visual memory but also for tactual memory. Though these studies suggested that the second somatosensory area (SII) and the insular cortex may act as relays for the transfer of somatic inputs from the first somatosensory area (SI) to the limbic system, it is known that, in addition to SII, a number of other fields in or near the lateral sulcus also receive somatic inputs. Because little is known about the connectivity or other properties of these fields, we have undertaken to study them with the goal of delineating (I) the route via which somatosensory information reaches the limbic structures critical for memory, (II) the corticocortical interrelations of the somatic fields involved, (III) the thalamic relationships of these fields, and (IV) their single-unit response properties.

The effects of drugs on brain functioning is one of the most important areas of current research. The limbic and diencephalic areas that we have shown to be important for learning and memory contain high levels of opiate receptors, and other workers have shown that opiates may alter learning ability under a variety of circumstances. As yet, however, the pathways by which opiate-containing neurons may project to the cortex are unknown, as are the specific pre- and post-synaptic effects of endogenous opiates. The initial stages of this project will attempt (V) to describe the opiate pathways that may affect learning and (VI) to relate the level of opiate-receptor binding to learning-related protein phosphorylation.

Methods employed:

Single and multi-unit recording techniques were used to identify the specific cortical fields in the lateral sulcus of the macaque that are activated by somatic input. These fields include the second somatosensory (SII) cortex, area 7b, the retroinsular cortex, and the granular and dysgranular insular fields. After a particular field was mapped, an injection of either tritiated amino acids (a mixture of proline and leucine) or HRP was made into the hand or digital representation within it to trace its connections. Accurate placement of the injection was ensured by either i) injecting through the recording pipette by iontophoresis or ii) recording from a microelectrode cemented to the needle of the injection syringe.

Histologic identification of cortical fields has been improved through processing of adjacent sections to reveal either cell bodies, with a standard Nissl stain, or axons, with a sensitive silver stain we have developed for bulk use.

Preliminary physiological studies of lateral sulcus neurons have been performed in immobilized monkeys, which were lightly anesthetized, and more complete studies are being performed in awake monkeys seated in a primate chair.

In transmitter studies, immunocytochemical and receptor binding techniques are being combined with more classical lesion and retrograde tracer methods. Opiatergic amygdaloid projections are being studied in monkeys that have had unilateral amygdalectomies. After sectioning on a cryostat, the tissue is incubated in tritiated ligands for mu and kappa receptors and processed for quantitative autoradiography.

Metabolic studies are performed on homogenized monkey brain tissue that has been dissected according to functional and cytoarchitectonic criteria and then assayed for receptor binding levels and for the rate of learning-related protein phosphorylation. Correlations of the levels of mu and kappa receptor binding and protein phosphorylation are then performed.

Major findings:

I. Corticocortical Connectivity:

Using the combined recording-injection techniques described above, we have placed injections into SII, area 7b, area 5, the retroinsular field (Ri), and the granular (Ig), dysgranular (Id), and agranular (Ia) insular fields. By combining the data concerning anterograde projections derived from the tritiated amino acid injections and retrograde projections derived from the HRP injections we have demonstrated reciprocal connections between: SII and Ri, SII and area 7b, SII and Ig and Id, and Ri and Ig. Also, we have confirmed previously reported reciprocal projections between SI and SII and demonstrated reciprocal projections between area 5 and both Ri and area 7b. Finally, anterograde labeling resulting from HRP injections into the insular

fields has confirmed recently reported projections from Ig and Id to the amygdala and from Id to the prorhinal and perirhinal cortical areas. These areas, in turn, send major projections to the hippocampus.

Our studies thus demonstrate that tactual information may reach the amygdala and hippocampus via relays in the granular and dysgranular insular fields, which receive their somatic cortical inputs from SII and Ri. A ventrally directed cortico-limbic pathway originating in SI may therefore be important for the perception and memory of somatosensory stimuli. This possibility is now being examined in a series of ablation studies.

II. Laminar Patterns of Termination:

Three different laminar patterns of terminal fields of the corticocortical projections described above were seen. Each pattern depended on the field into which the injection was made and the field to which the injected field projected. Though similar patterns have been described in other areas of the cortex, only one has previously been reported in the somatosensory system.

This pattern consists of a heavy band of labeled terminals in layer IV, with progressively lighter labeling, indicative of fewer terminals, in the supragranular layers, III, II, and I. There is a light band of terminal labeling in layer VI paralleling that seen in layer IV. This pattern has been described for the projections from SI to SII and area 5, and from area 5 to Ri and 7b. It is similar to the forward (i.e. outward from striate cortex) projections seen in the visual system.

The second pattern is analogous to the one described in the visual system as a backward projection (i.e. towards the striate cortex). Its most striking characteristics are a complete absence of labeling in layer IV and heavy labeling in layer I. Additional labeling is seen in layer VI and sometimes in layers III and II. The projections from SII to SI and Ri, from Ri to area 5, and from Ig to SII and Ri are all of this type.

The third pattern, previously described in prefrontal association areas, consists of a single, apparently homogeneous column of labeled terminals extending from layer VI through layer I. Its most striking feature is the lack of laminar differences in labeling density, in sharp contrast to the so-called forward and backward projections described above. This pattern is seen in the projection from SII to area 7b.

By analyzing the pattern of forward and backward projections, we have been able to determine the probable sequential order in which information is processed in the somatosensory system. The forward direction is SI to SII and area 5, area 5 to Ri and area 7b, area 7b and Ri to SII, and SII and Ri to Ig. SII also projects to Id. Ig and Id then project to the limbic system.

III. Thalamocortical relations:

In conjunction with the above work, the thalamic connectivity of the cortical fields of the somatosensory system was thoroughly examined. This study was required because preliminary findings indicated that the thalamic relations of these cortical fields differ from that described in the literature. By having, for the first time, an appreciation of the full extent of these fields, and by using our combined recording-injection techniques to increase the accuracy of our injections, we have been able to provide a new account of this fundamental anatomical relationship.

There are three major new findings: (1) The second somatosensory area (SII) receives its major thalamic input from the ventroposterior inferior thalamic nucleus (VPI) and additional inputs from the central lateral nucleus and possibly from the parvocellular division of the mediodorsal nucleus as well. Previously reported inputs from the caudal division of the ventroposterior lateral nucleus (VPLc) could not be confirmed, and if they exist, they may arise only from the most ventral and caudal portions of VPLc and from the ventroposterior medial nucleus (VPM). The finding in the monkey that SI and SII receive different thalamic inputs is consistent with the hypothesis that they process information in a sequential rather than parallel manner, the latter notion having been based on previous reports that both fields received their thalamic input from VPLc. Because of the importance of determining whether the nature of cortical processing is sequential or parallel we are pursuing this with additional experiments (see proposed course, below). (2) The dysgranular insular field (Id) receives thalamic input from a continuous band of neurons that runs caudally and ventrolaterally from the basal ventromedial nucleus (VMB) through VPI and the posterior nuclei (Po) to the medial edge of the medial geniculate body (MG) and not simply from VPI as previously reported. Additional inputs to Id arise from the intralaminar nuclei and nucleus reuniens and from the medial nucleus of the pulvinar (Pulm). (3) Pulm projects to a number of the cortical somatosensory fields including Id, the granular insula (Ig), and area 7b. Thus, the cortical area receiving projections from the medial pulvinar includes not only the temporal lobe, as previously reported, but also the lateral sulcus and parts of the parietal lobe. This raises questions to be explored regarding the nature and function of such a widespread projection.

We have confirmed projections to Ig from the suprageniculate and limitans nuclei and to the retroinsular area from the posterior group. However these projections do not conform to previous descriptions because the thalamo-cortical relay neurons labeled retrogradely by HRP injections into a single cortical field appear to cross the borders of thalamic nuclei, as, for example, in the case of the projection to Id described above.

These findings suggest a revision of the traditional view of thalamo-cortical organization, which states that a single thalamic relay nucleus projects to a single cortical field. In the somatosensory system, at least, it now appears that each cortical field outside of SI receives inputs from a number of

thalamic nuclei and that each thalamic nucleus projects to several cortical fields.

IV. Physiological Studies of Insular Cortical Neurons:

We have examined in an awake monkey a population of cells located in the posterior two-thirds of the insula, comprising the granular (Ig) and dysgranular (Id) insular fields. Of 184 neurons studied in this region, 181 received somatic input; none was found that responded to visual input, one had auditory input and two had gustatory inputs. The somatosensory neurons of the insula most frequently have bilateral receptive fields (128/181), though a small number are more responsive to stimulation on the contralateral than on the ipsilateral side (22/128). Most receptive fields (162/181) are larger than those found in SI or SII without extending over disparate regions of the body. About 1/4 of the units activated by limb stimulation, however, did have receptive fields that included the entire limb (19/78). Inputs from both cutaneous (143 units) and deep (38 units) receptors are represented in the insula, but there is no evidence that there is convergence of these modalities onto single units.

The submodality distribution in the insula (77% cutaneous, 23% deep) is close to that of SI (73% cutaneous, 27% deep) and SII (73% cutaneous, 17% deep, 8% other), and many cutaneous properties of SI and SII neurons (e.g. discrete receptive fields, low thresholds, no convergence) may be found. The insula does differ markedly from SI, however, in the bilaterality of the receptive fields of its neurons. Moreover, given its large number of undriven cells (37%), many of its neuronal properties are still unknown. By analogy with higher cortical stations in the visual system, we would expect that complex somatic stimuli, or those with particular relevance for the animal, may be necessary to drive these cells.

The insula is clearly unlike either area 5 or 7b. Convergence of joint and skin submodalities or of inputs from several joints is common in these latter areas, but not in the insula. Moreover, about 90% of the cells of area 5 are related to the contralateral side of the body, whereas about 70% of the cells of the insula have bilateral receptive fields. Finally, convergence of somatic and visual inputs, which is a characteristic of area 7b, is absent in the insular neurons studied here. Interestingly, however, a few units (10/181) did increase their activity when the animal looked at the object which was delivering tactile stimulation and decreased their activity when the animal closed its eyes. These influences may reflect some type of attentional process.

For some insula neurons (14/181), the coupling between peripheral stimulus and neural discharge seems weak in comparison with the tight coupling of most of the cells in the first and second somatosensory areas and in area 5. However, we found many low threshold cells in the insula (39%). The ratio of slowly adapting to rapidly adapting units is small (6/49). Finally, there may be a somatotopy among the cells of the insula. It seems to be organized with the most dorsal portions of the insula (medial on the cerebral cortex) containing

the representation of the rostral parts of the body, and the most ventral portions of the insula (lateral on the cerebral cortex) containing the representation of the caudal body parts. This somatotopy is crude in relation to that seen in SI or SII but, due to our relatively restricted sample of neurons, this conclusion must be considered only as preliminary.

V. Opiatergic Projections of the Amygdala:

Analysis of [^3H] naloxone binding levels has been performed on sections from one monkey brain that had received a unilateral amygdalectomy 30 days prior to sacrifice. The sections show an increase in naloxone binding ipsilateral to the lesion in the anterior portions of the insula, which projects to the limbic areas of the temporal lobe, and in regions of the orbitofrontal cortex that appear to be required for memory formation. This increased binding is interpreted as a denervation supersensitivity of mu receptors following amygdalectomy, and suggests that the amygdala does indeed send an opiate projection to the brain regions mentioned. We are now attempting to replicate this finding in additional monkeys and to extend the analysis to other regions of the brain.

VI. Metabolic Studies:

In order to correlate the levels of opiate binding with levels of learning-related protein phosphorylation, correlations of naloxone binding levels and protein phosphorylation rates have been carried out in two monkeys. The results demonstrate: (a) an inverse correlation across 22 brain regions between the density of [^3H] naloxone binding sites and the in vitro phosphate incorporation in the 45kD band, Fl ($r=0.610$, $p .01$); (b) the presence of a 47 kD protein with properties like the 45 kD band only in those regions of highest naloxone binding; and (c) a positive correlation between the phosphorylation of a 49kD protein and [^3H] naloxone binding levels ($r=0.564$, $p .01$). These findings suggest a local control of protein phosphorylation in monkey cerebral cortex corresponding to opioid receptor levels and indicate that opioid peptides may in fact help control metabolic processes underlying memory formation.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Studies concerning the connections of the somatosensory system have been relatively restricted in scope. This project supplies the first comprehensive look at the entire somatosensory system and how it may connect with the limbic structures necessary for memory. Furthermore, this project is yielding fundamental insights into how the cerebral cortex processes information by describing the precise laminar pattern of connections and by adding new data about the thalamic connections of these fields. As a whole, our studies have demonstrated remarkable parallels between the organization of the somatosensory and the visual systems, suggesting that common mechanisms of perception and memory operate within both, and that further studies of each one will illuminate the other.

Knowledge about the functions of specific transmitter systems has both basic and applied value. Descriptions of the opiate pathways will supply the fundamental descriptive information needed to help understand brain functioning and will help guide behavioral experiments by supplying targets for lesion experiments. The studies of protein phosphorylation may supply information concerning the mechanisms by which the opiates affect neuronal metabolism in general and learning specifically. Eventually, this may lead to therapeutic advances in the treatment of memory and learning disorders.

PROPOSED COURSE OF RESEARCH:

To follow up our preliminary findings we next plan to train monkeys in tactile recognition tasks and to record from insula neurons during such tasks. We hope to be able to activate the large number of insula neurons which remained silent during the passive application of tactile stimuli in our current study.

To test further the hypothesis that somatic information is processed in a sequential manner through the cortical fields of the somatosensory system, we are preparing animals with removals of the first somatosensory area and chronic recording chambers that will allow us to record from the second somatosensory area and from the insula. If the somatosensory system is organized like the visual system, we expect that SII will become silent, demonstrating its dependence on SI for somatic information.

In the transmitter studies the major goals are to provide details concerning the location of various transmitters and neuropeptides that have been shown to have an effect on learning and memory. First, we will replicate and extend the current findings with additional monkeys that have already been prepared. In order to provide further evidence that opiate neurons in the amygdala do project to specific cortical areas, double labeling experiments involving these pathways will be carried out. Injections of horseradish peroxidase will be made into the areas that show increased naloxone binding after amygdectomy. The sections containing the retrogradely labeled neurons will then be incubated with enkephalin antibodies to demonstrate endogenous enkephalin in the retrogradely labeled neurons. If successful, this technique may be used to demonstrate the cell bodies of origin and terminal fields of the major enkephalinergic pathways that affect learning and memory.

The metabolic studies will also be continued in an attempt to refine the initial findings. Attempts to determine the relation of kappa receptors to the current system will be undertaken and further efforts to define the exact nature of the phosphoprotein will continue.

A third major goal is the localization of those neuropeptides that have been shown to have an effect on learning and memory (e.g. alpha-MSH). The locations of the cell bodies and terminals containing these putative transmitters will be determined in the monkey as a guide for behavioral experiments.

PUBLICATIONS:

Friedman, D.P.: Laminar patterns of termination of cortico-cortical afferents in the somatosensory system. Brain Research (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02038-01 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Ontogenetic development of memory and habit formation		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jocelyne Bachevalier, Guest Worker, LN, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Memory formation and habit formation</u> are two qualitatively different retention processes based on <u>separate neural mechanisms</u>. Recent <u>developmental studies in monkeys</u> have indicated that these two systems of retention are developmentally dissociable, with the <u>nonlimbic habit system</u> maturing earlier than the <u>limbic memory system</u>. On the evidence that the limbic (i.e. amygdalo-hippocampal) memory system is essentially nonfunctional in infants, we have begun to prepare monkeys with neonatal removal of this system to see how <u>cognitive, emotional, and social behavior</u> develops in animals whose <u>amnesia</u> might persist from infancy through adulthood. Animals with <u>neonatal removal of area TE</u>, a higher order station of the visual system, served as controls. The results so far indicate that, at two months of age, monkeys with <u>neonatal limbic lesions</u> display abnormal social behavior, whereas the operated controls are essentially unimpaired relative to normal infants. Similarly, at three months of age, the infants with limbic lesions are impaired in habit formation (even though the same lesion in adulthood leaves the habit system intact), whereas the operated controls showed significant functional sparing of the system (compared to the effects of TE lesions in adults). Our tentative conclusion is not only that early and late brain damage have different consequences but also that the direction of the difference depends on whether the damage is cortical or subcortical: Early cortical damage may be less disruptive than late, while early subcortical damage may be more disruptive. </p>		
(567)		

Principal Investigator:

J. Bachevalier Guest Worker

LN NIMH

Other Investigators:

M. Mishkin Chief, Laboratory of Neuropsychology LN NIMH

Technical Support Staff:

H.T. Crawford Psychology Technician LN NIMH

PROJECT DESCRIPTION

Objectives:

Findings from studies of the effects of lesions in adult monkeys suggest that memory and habit formation are qualitatively different retention processes based on separate neural mechanisms. The memory system, which serves both recognition and associative memory, utilizes a cortico-limbo-diencephalic circuit. By contrast, the habit system, which mediates retention of stimulus-response connections, probably depends in large part on a cortico-striatal system. Our recent studies of behavioral development in infant monkeys have suggested that these two systems are developmentally dissociable, in that the nonlimbic habit system appears to mature considerably earlier than the limbic memory system. On the evidence that the limbic memory system is essentially nonfunctional in infants, we have begun to prepare monkeys with neonatal removal of this system in an attempt to see how cognitive, emotional, and social behavior develop in animals whose amnesia might persist from infancy through adulthood.

Infant rhesus monkeys received damage to either the limbic system, i.e. amygdalo-hippocampal complex, or the anterior part of inferior temporal cortex, i.e. area TE. Both lesions are known to produce severe impairment of visual memory in adult monkeys. The bilateral lesions were performed in two unilateral stages at approximately one week and three weeks of age, respectively. Three factors dictated selection of the TE lesion as the control operation: (a) whereas amygdalo-hippocampal removals in adult monkeys impair the ability to form new memories but not the ability to acquire new habits, TE lesions in adults produce a severe impairment in both forms of retention; (b) conversely, disturbances in social behavior have been observed after limbic but not after TE lesions in adulthood; and (c) since both area TE and amygdalo-hippocampal lesions produce impairments in visual memory, the use of these two types of lesions permit comparison between the effects of neonatal cortical versus neonatal subcortical lesions on the development of visual memory processes. Each experimental and operated control animal was age-matched with a normal monkey. We plan to follow the behavior of these

animals from birth to five years of age in order to assess the effects of neonatally-induced amnesia on (1) the maturation of cognitive functions and skill learning, as measured by a variety of visual memory, problem solving, and habit formation tasks, and (2) the development of emotional and social behaviors, as measured by interactions with familiar vs. unfamiliar and normal vs. operated monkeys of both sexes and various ages, and by reactions toward familiar vs. unfamiliar and emotionally neutral vs. emotionally challenging environments and stimuli.

Major Findings:

To date, we have operated four monkeys with bilateral amygdalo-hippocampal lesions and six with bilateral TE lesions. These monkeys were age-matched with eight normal animals and have already undergone some testing for social behavior and learning abilities. At two months of age, three animals with limbic lesions and four with TE lesions were tested for social behavior in two different situations: (a) the operated animal and its age-matched normal control were caged separately but adjacently so that the two monkeys could reach and touch each other; and (b) the two monkeys were placed together in a test cage containing plastic toys and towels. The animals with limbic lesions displayed a moderately greater frequency of motor activity, stereotyped behavior, and self-directed activity than either of the control groups in both situations. In contrast, the monkeys with TE lesions did not differ from normal infants in either situation. At three months of age, the animals were trained in the 24-hour concurrent object discrimination learning task, a sensitive measure of habit-formation ability. Unlike animals given limbic lesions in adulthood, who perform as well as normal adults, the three infants with limbic lesions demonstrated a mild retardation in acquisition of the task. Conversely, unlike animals given TE lesions in adulthood, who show a severe deficit in acquiring the task, the infants with TE lesions were only slightly impaired during the first phase and unimpaired during the second, performing as well as both normal infants and adults. The findings with the normal controls support earlier data showing that habit formation proceeds at adult rates in early infancy. However, the results with the operated animals suggest that, although area TE is involved in habit formation in adults, neonatal ablation of this cortical area leads to significant functional sparing of this ability; conversely, although limbic lesions in adults leave habit formation intact, neonatal limbic ablation leads to an impairment in the ability.

These preliminary findings suggest that the consequences of early neural damage may be different from those of the same damage in adults and, further, that the direction of the difference may depend on whether the locus of injury is cortical or subcortical. It is of course still too early in this long-term project to form any strong conclusions, but the findings to date do provide evidence of the practicability and potential value of the study.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

Developmental studies of the effects of early brain damage are of great importance for the assessment and understanding of those errors of central nervous system maturation that cause children to become autistic, dyslexic, learning disabled, or mentally retarded. This project will supply the first comprehensive investigation of social and cognitive development of monkeys suffering from an amnesia induced early in infancy. It will thus permit comparisons of the cognitive and social behavior of the infant-operated animals with those of animals who have sustained the same lesion in adulthood i.e. after memories have been formed and consolidated in cerebral tissue outside the limbic system. In addition, comparison of the effects of early cortical and subcortical lesions will help answer whether or not compensatory mechanisms always operate to promote recovery from early brain injury. Our preliminary results suggest otherwise. Finally, in assessing the effects of early and selective temporal-lobe damage on infant, juvenile, and adult behavioral patterns, this project will help to evaluate two provocative proposals from the clinical literature: (a) that early dysfunction of the limbo-thalamic memory system is one cause of childhood autism, a syndrome characterized by dramatic social and emotional disturbances not seen in adults with the same neuropathology; and (b) that the reason a pure case of anterograde amnesia like the one seen in adults has never been reported in a child is that the clinical picture of an amnesic child, being overlaid with autism, is entirely different from the clinical picture of an amnesic adult.

PROPOSED COURSE OF RESEARCH:

Our goal is to continue our examination of the effects of neonatal limbic lesions on social and emotional behavior as well as on memory and learning at several periods throughout development from infancy to adulthood in order to test whether such a preparation does indeed provide an animal model of childhood autism. We shall also initiate studies to determine whether the learning capacities measured by habituation tasks are mediated by the memory system or the habit system. This latter project will help test whether the memory system does indeed appear late in ontogenetic development and, by implication, whether the phenomenon of infantile amnesia is due to the absence of a functional memory system in early childhood.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02039-01 LN
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cholinergic mechanisms in memory		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Thomas G. Aigner, Staff Fellow, LN, NIMH		
COOPERATING UNITS (if any) The Johns Hopkins University, Baltimore, MD		
LAB/BRANCH Laboratory of Neuropsychology		
SECTION		
INSTITUTE AND LOCATION NIMH, NIH, Bethesda, MD 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>nucleus basalis of Meynert (nbM)</u> is the major source of <u>cholinergic projections</u> to widespread areas of the neocortex. In <u>Alzheimer's disease</u>, which is often accompanied by memory loss, there is evidence for a selective loss of neurons in this nucleus. To determine the involvement of the nbM in memory in monkeys, this nucleus was destroyed with the neurotoxin, <u>ibotenic acid</u>. Although performance of a <u>recognition memory</u> task was unimpaired by the lesion, the operated animals were found to be more sensitive to the effects of <u>scopolamine</u> and less sensitive to the effects of <u>physostigmine</u> than were their unoperated controls. The results suggest that destruction of nbM reduced but did not eliminate the basal forebrain cholinergic system and thereby rendered it both more vulnerable than normal to memory disruption by a cholinergic antagonist and less responsive than normal to memory enhancement by a cholinergic agonist. </p>		

Principal Investigator:

T.G. Aigner Staff Fellow

LN NIMH

Other Investigators:

J. Aggleton Visiting Fellow

LN NIMH

S. Mitchell Research Associate

The Johns Hopkins Univ.

M. DeLong Medical Officer/Professor

The Johns Hopkins Univ

M. Mishkin Chief, Laboratory of Neuropsychology

LN NIMH

Technical Support Staff:

S.K. Presty Research Assistant

The Johns Hopkins Univ.

PROJECT DESCRIPTION:

Several lines of evidence have suggested that cholinergic mechanisms are involved in normal memory functioning. Thus, cholinergic agonists and antagonists are known to enhance and impair memory abilities, respectively. Further, a decrease in the activity of the cholinergic system may be responsible for the memory loss observed in aging and in Alzheimer's disease. For example, choline acetyltransferase and acetylcholinesterase, two enzymes involved in the synthesis and metabolism of acetylcholine, have been reported to be decreased in the cortex and hippocampus in patients who have died from Alzheimer's disease. In addition, several recent studies have reported that in such patients the nucleus basalis of Meynert (nbM) and the diagonal band of Broca (dbB), the major sources of cholinergic projections to the neocortex, show evidence of selective degeneration. Finally, neurons in these nuclei have also been shown to project to the hippocampus and amygdala, structures that are known to be critical for memory function.

Experiment 1:

In a preliminary study, we evaluated the effects of two classic cholinergic drugs, scopolamine and physostigmine, on recognition memory in normal rhesus monkeys. The animals were first trained to perform a delayed nonmatching-to-sample (DNMS) task in a Wisconsin General Testing Apparatus. For this study, the animals were shown 20 objects one at a time. These objects were then each paired with 20 novel objects, and the animal was required to choose the new object to obtain a reward. Each daily test consisted of 2 such series of 20 trials each, or 40 trials per day. After the animals were reliably performing this task, the effects of several doses of scopolamine or physostigmine were evaluated. The results showed that scopolamine, a muscarinic receptor blocker, produced a dose-related decrease in the number of objects correctly remembered. Conversely, physostigmine, a reversible cholinesterase inhibitor, produced a dose-related increase in

correct responses. These results indicated that the DNMS task was a sensitive procedure for studying the effects of both drugs and lesions of the cholinergic system on memory in monkeys.

Experiment 2:

In order to test the role of the nbM cholinergic system in memory, a new group of cynomolgus monkeys was trained on the DNMS task just described. Following initial training, the animals were surgically prepared with bilateral lesions of the nbM by injection of ibotenic acid, a neurotoxin previously shown to destroy neuronal cell bodies without harming fibers of passage. The location of the nbM was identified by electrophysiological recording techniques. After being allowed 2 weeks for recovery, the animals were retested on the DNMS task. Two of the monkeys relearned the task in the minimal number of trials (100), while the third animal required an additional 20 trials to reach criterion. The animals were then given a series of performance tests involving delays of 30, 60, and 120 seconds and then lists lengths of 3, 5 and 10 objects to be remembered. In general, the results appeared to indicate that ibotenic acid lesions of the nbM were without significant effect.

Nevertheless, in order to test the effects of the nbM lesions in these animals even more rigorously, the effects of several doses of scopolamine and physostigmine were evaluated. For the drug tests, the animals were required to remember a list of 20 objects. Two unoperated cynomolgus monkeys, with similar behavioral histories, served as controls. Scopolamine produced a dose-related decrease in the number of objects correctly remembered by both groups of monkeys. However, at each dose tested, the operated animals made more errors than the control monkeys. Conversely, while physostigmine increased the number of correct responses in both groups, the effect was greatly attenuated in the animals with the lesions.

Experiment 3:

In order to determine if more complete lesions of the forebrain cholinergic system would produce more reliable memory impairments, a third study has been started. To date, a single cynomolgus monkey has been trained on the DNMS task and surgically prepared with an ibotenic-acid lesion that includes not only the nbM but also the medial septal/diagonal band nuclei, regions known to send cholinergic inputs to the hippocampus. Unlike the previous animals, this one was severely impaired in relearning the task, requiring 1040 trials to reach criterion. The animal also showed a severe impairment when 120-second intervals were imposed, making only 67% correct responses.

SIGNIFICANCE TO MENTAL HEALTH RESEARCH:

In these studies we are examining the effects of selective, neurotoxin-induced lesions of the cholinergic system on memory functioning in monkeys. If present trends continue, we will have succeeded in developing an extremely valuable animal model of the type of memory loss that is associated with

Alzheimer's disease. Such a model would allow us not only to study the effects of manipulating the cholinergic system, but also to evaluate possible therapeutic approaches to diseases involving cholinergic deficiencies.

PROPOSED COURSE OF RESEARCH:

Our results to date suggest that large ibotenic-acid lesions involving both the nbM and the medial septal/diagonal band nuclei are required to produce significant memory deficits in monkeys. We are now in the process of checking the reliability of that result with additional monkeys given this combined lesion and others given smaller lesions restricted to the medial septal/diagonal band nuclei alone. These groups will be evaluated in the same way as the first group of monkeys, both behaviorally and pharmacologically. At the completion of testing, all monkeys will be sacrificed and the brains prepared for analysis of choline acetyltransferase activity in specific tissue samples and for histological verification of lesion size and location. An attempt will also be made to correlate any changes in enzyme activity with the results of behavioral and pharmacological manipulations.

Z01 MH 00471-28 LPP

October 1, 1982 to September 30, 1983

Studies of Heredity and Environment in Schizophrenia

Allan F. Mirsky, Chief, LPP, NIMH

Institute for Research on Kibbutz Education; William Beaumont Hospital, Michigan; and Boston University

Laboratory of Psychology and Psychopathology

NIMH, ADAMHA, Bethesda, Maryland 20205

0.5

☒ (a2) Interviews

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. (2) Studies of adoptees and their biological and adoptive families. (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

Other Professional Personnel

Edward K. Silberman, M.D., Guest Worker, LPP, NIMH
 Shmuel Nagler, Ph.D., Research Psychologist, Institute for Research on Kibbutz Education (Israel)
 Olive W. Quinn, Ph.D., Guest Worker, LPP, NIMH
 Patricia Lowing, Ph.D., Staff Psychologist, William Beaumont Hospital
 Arje Latz, Ph.D., Associate Professor, Boston University

Project Description

The project is composed of the following studies: (1) An intensive multi-disciplinary study of a family with MZ quadruplets (daughters) concordant as to schizophrenia but discordant as to severity and outcome. We are continuing our contacts with this family to see what happens in the clinical course of these women and to see how the course is related to earlier and to current life experiences; (2) Studies of adoptees and their biological and adoptive families in Denmark; (3) A study of children (of schizophrenic and control parents) reared in town or kibbutz in Israel.

The objectives of this project are to understand how hereditary and environmental factors interact to make for schizophrenic outcomes of varying types and degrees.

1. The Genain Quadruplets

In 1963 David Rosenthal published the results of an extensive study of a group of four women, identical quadruplets, all of whom had succumbed to schizophrenic illness at some point during their late teens or early twenties. The women (who were named by Rosenthal for this publication: Nora, Iris, Myra, and Hester, i.e., N.I.M.H.) were studied by a group of psychologists and psychiatrists at the NIMH and were examined with virtually all of the methods extant in the late 1950's for studying schizophrenia and psychological deficit. After a period of study at the NIMH which extended over several years and which relied heavily on psychiatric treatment of the dynamic variety (both as therapy and as a means of gaining information), Rosenthal summarized the investigative effort in the Genains by the suggestion that the diathesis-stress theory was a reasonable way of accounting for the differences in the severity of their psychiatric illness. Although they shared an identical heredity (diathesis), differences in the way they were treated by their parents and significant others in their environment led to different expectations and self-pictures and consequently to different phenotypic expression of the schizophrenic disease. The more competent "pair", Nora and Myra, were more favored and fussed over; the smallest and least prepossessing physically, Hester, was most often bracketed with Iris. Willy-nilly, Hester and Iris were treated as the less competent and capable pair and more or less fulfilled that expectation. This is a somewhat oversimplified but reasonably accurate summary of the earlier view of the Genains.

Rosenthal and his early colleague and collaborator, Olive Quinn, maintained contact with the Genains and with their mother (who died this year at age 84).

Through Rosenthal's and Quinn's good offices and contacts, we were able to persuade the Genain clan to return to NIMH for another period of study. In addition to the quadruplets themselves, the group included the mother, the husband of Myra (she is the only one to have married) and Myra's two adolescent sons. On this occasion, which lasted for a period of approximately 3 1/2 months, we tested the Genains with the full battery of neurobiological test procedures that have evolved over the last 25 years. The procedures included: an extensive series of genetic identity tests; biochemical determinations from blood, urine, and cerebrospinal fluid of various catecholamine compounds with emphasis on dopamine and norepinephrine; procedures related to the identification of possible preexisting viral infection of the central nervous system; neuroradiological and neurophysiological tests (CT scan, PETT scan, evoked potential and EEG brain maps, brain stem evoked potentials); and an exhaustive battery of psychological and psychometric tests with a special focus on measurement of attention, arousal and memory. Two of the tests were essentially identical to measures employed in the late 50's--the continuous performance test and the reaction time paradigm. Further, for most of the behavioral tasks, we were able to examine the Genains both on and off medication--the latter after a period of at least two weeks free from the phenothiazine drugs they were taking on admission to the NIMH.

We conclude that the Genains are functioning about as well as they ever have in their adult lives, and scores on attention tests show improvement as compared with 1958 measures. This is likely attributable to the medication (primarily phenothiazines) and other supportive treatments they have received over the years. With respect to the varying degrees of illness seen in the Genains, the following findings appear relevant: the tests indicate that two of the women (Nora and Hester) deteriorated rapidly when removed from medication, and two (Iris and Myra) did not. The consequence of this is that the grouping of the quadruplets on the basis of their characteristics and abilities while they are medicated is different from that apparent while they are off medication. On medication the apparent pairing is Nora and Myra (as before) and Hester and Iris. Scrutiny of the test material, including the biochemical, physiological, neuroradiological and immunogenetic, as well as behavioral, leads to speculation that certain unique biochemical findings and differing types and amounts of cerebral pathology may constitute the fundamental cause of the variable expression of schizophrenia in the Genains. This set of circumstances is superimposed on a basic schizophrenic diathesis which is manifest in the biochemical and certain neurological and neurobehavioral findings. The interdisciplinary research effort represented by this series of studies is unique in the annals of schizophrenia research and has led, we believe, to testable hypotheses on the role of various neurobiological factors in the development, etiology, and expression of the disease.

A series of three studies with first authors respectively, Lynn Delisi, Monte Buchsbaum and Allan F. Mirsky, have been submitted for publication to Psychiatry Research.

2. The Danish Adoptee Study--Reanalysis of the Data

Using data from Danish health records, in a now-classic study, Rosenthal, Kety

and Wender compared the frequency of schizophrenia spectrum disorders in two groups of persons adopted in infancy or early childhood: those with a psychotic parent (index group) and those whose biological parents had never had psychiatric treatment (control group). Significantly more disorder was found in the index than the control group. This study has been criticized recently on the grounds that subjects were included inappropriately (affective rather than schizophrenic diagnoses in the parents; insufficient information available about the father). We have completed a reanalysis of the original material using the new DSM III methods, and stricter exclusionary criteria applied to the parents. The results of the reanalysis yielded three times as many schizophrenia spectrum disorders in the index as in the control group, a slightly better result than that found in the original Rosenthal et al., study. The difference between groups remains statistically significant, supporting the operation of genetic factors in the transmission of schizophrenia spectrum disorders. The manuscript describing the findings is in press in the American Journal of Psychiatry. A second manuscript describing the relation (in these same subjects) between reported stress (in childhood) and severity of schizophrenia spectrum illness is being prepared for publication.

(3) The Israel Kibbutz--High Risk Study

During the past year, the Laboratory has been engaged in completing work begun in 1962 on the study of children at risk for schizophrenia in Israel, which was designed and initiated by Dr. David Rosenthal. The study has examined 100 children, of whom 50 had one schizophrenic parent, and 50 were born to two nonschizophrenic parents. Half of both "index" and control groups were reared in towns in traditional nuclear families, while the remaining half were reared in communal settings on kibbutzim.

Our work has been in two phases. The first has been to complete data analysis of the initial examination of subjects, done when they averaged 11 years of age, and a major portion of the second examination when they averaged 17 years of age, and prepare manuscripts for the first major publication of the results. It is not easy to present the problems involved in executing and completing a study involving this much international collaboration. There have been enormous technical difficulties in getting the manuscripts from the several Israeli collaborators, sending revisions back and forth and securing approval for various publication plans. Only this year (1983) has it been possible to secure and revise the last of the manuscripts. At the present time, data analysis is complete, and manuscripts are in the final stages of completion (for publication in the Schizophrenia Bulletin). In broad outline, the results indicate that index children were discriminable from controls in many areas of function, but kibbutz and town children did not differ on the experimental examinations. Furthermore, kibbutz versus town rearing had no discernible effect on the performance or behavior of high-risk children. Index children were found to be poorer in psychosocial adjustment, perform more poorly in school, manifest a number of neurological "soft signs", and show deficits on psychological tests requiring high levels of attention, visual integration, and visuo-motor coordination. An important negative finding was lack of differences between index and control

children on psychophysiological measures of arousal and habituation in the first examination. Recent information (which will not be included in the Schizophrenia Bulletin publication) indicates that the lack of psychophysiological difference was sustained in the second examination.

The second phase of the study has involved the collaboration of Dr. Arje Latz, of Boston University, who has been engaged in conducting follow-up interviews with study subjects. These subjects are now in their mid-twenties, at the peak of their risk period for schizophrenic breakdown. Ninety of the surviving 99 subjects have been seen at this writing. Results show that nine subjects fall within the "schizophrenia spectrum" (of whom six are DSM III schizophrenic), six from kibbutz backgrounds, and three from towns. When all DSM disorders are considered, more than five times as many ill subjects fall within the index (N=23) as within the control group (N=4). Furthermore, when schizophrenia itself is excluded, the remaining subjects with history of illness (including DSM III Major Affective Disorder or Dysthymic Disorder) are found predominantly in the index-kibbutz cell (16 of the total of 27 in the cell). Other significant preliminary results include persistence of attention-related deficits in the index group, and continued poor social and work adjustment in high-risk subjects. Some of these outcome results are being prepared for possible publication in Science.

At present, work on the project centers around completion of data collection for the adult follow-up study. These data will then be compared to information from the previous cross-sectional studies with this group in an attempt to elucidate possible precursors of adult illness.

Significance to Biomedical Research and to the Program of the Institute

The issue of the mode of heritability of mental illness, and factors which modify it, may be the highest priority of the Institute. This work contributes significantly to our knowledge in this area and ultimately, to our capacity to treat and prevent schizophrenia and related disorders.

Proposed Course

The remaining data analyses will be completed and the work will be prepared for publication. No new data gathering is planned at this time. It is estimated that one more year will be necessary for the completion of this work.

Publications

Rosenthal, D., Nagler, S., Silberman, E., Mirsky, A.F. and Kugelmass, S. (Eds.): The Israeli High Risk Study. Schizophrenia Bulletin, in preparation.

Lowing, P.A., Mirsky, A. F. and Pereira, R.: The inheritance of schizophrenia spectrum disorders: A reanalysis of the Danish adoptee study data. Am. J. Psychiatry, in press.

Mirsky, A. F. and Duncan-Johnson, C.: Nature versus nurture in schizophrenia--the struggle continues. Integrative Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00472-20 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Investigations of Some Formal Characteristics of Speech		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Theodore P. Zahn, Ph.D., Research Psychologist, LPP, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.0	PROFESSIONAL: 0.0	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> This research project investigates <u>attention</u> during natural <u>speech perception</u>. Specifically, we are attempting to test the hypothesis that speech perception involves phasic, rather than continuous attention, and is characterized by bursts of cognitive activity at linguistically specified boundaries in the stream of speech. The method involves the measurement of <u>reaction time</u> to irrelevant stimuli--clicks--while the subject is listening to a <u>tape-recorded</u> dialogue. If the hypothesis is correct, the responses should be relatively inhibited for those clicks timed to occur during the postulated bursts of cognitive activity at linguistic boundaries. </p> <p style="margin-top: 20px;"> This project was temporarily suspended during FY 1982 and is now terminated. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00484-23 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychophysiologic Responsivity and Behavior in Schizophrenia		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Theodore P. Zahn, Ph.D., Research Psychologist, LPP/NIMH		
COOPERATING UNITS (If any) Laboratory of Socio-Environmental Studies, Laboratory of Clinical Science, Clinical Neuropharmacology Branch, Neuroscience Branch, and Biological Psychiatry Branch, NIMH		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.7	PROFESSIONAL: 0.8	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The general purpose of this project is to investigate the roles of <u>autonomic nervous system (ANS) activity</u>, <u>attention</u>, and <u>information processing</u> and their inter-relationships in the pathology, etiology, and prognosis of psychiatric disorders. A second purpose is to determine biological and psychological processes related to ANS activity. ANS activity is assessed by peripheral measures, such as <u>skin conductance</u>, <u>heart rate</u>, and <u>skin temperature</u>. Subjects with diagnoses of <u>schizophrenia</u>, <u>depression</u>, and <u>neurosis</u> are tested under conditions of rest, presentation of tones, and performance on <u>reaction time</u>, <u>mental arithmetic</u>, <u>two-flash discrimination</u>, or <u>tachistoscopic recognition</u> tasks. Biological mechanisms influencing ANS activity and attention are investigated by testing the effects of drugs and other treatments and by correlating these variables with enzyme activity and levels of biogenic amines and their metabolites. Psychological determinants are investigated by correlating the results with personality, mood, and personal history questionnaires by information from interviews, and by the effects of procedural variations. </p> <p> Studies are being done on unmedicated patients with diagnoses of <u>schizophrenia</u>, <u>affective disorder</u>, <u>obsessive-compulsive disorder</u>, <u>anxiety-panic disorder</u>, and <u>autism</u> to test the diagnostic specificity of patterns of ANS activity. Effects of state changes are studied in cases of <u>multiple personality</u> and in women in different phases of the menstrual cycle. Effects of <u>pimozide</u>, <u>GHB</u>, <u>propanolol</u> and <u>hemodialysis</u> have been studied in schizophrenics. Obsessive patients have been studied while taking <u>clorgyline</u> and <u>clomipramine</u>. The use of confirmatory factor analysis in data reduction and to improve quantification of ANS activity is being explored. </p>		

Other Professional Personnel

Theodore P. Zahn, Ph.D., LPP, NIMH
 Carmi Schooler, Ph.D., LSES, NIMH
 Dennis Murphy, M.D., Chief, CNB, NIMH
 David Pickar, M.D., NSB, NIMH
 Thomas Uhde, M.D., BPB, NIMH
 David Rubinow, M.D., BPB, NIMH
 Thomas Robinson, Jr., Ph.D., LPP, NIMH
 Daniel Hommer, M.D., NSB, NIMH
 Judith Rumsey, Ph.D., LCS, NIMH
 Judith Rapoport, M.D., LCS, NIMH
 Thomas, Insel, M.D., CNB, NIMH

Project DescriptionA. Objectives

The major objective of this project is the further understanding of the role of autonomic nervous system (ANS) activity, information processing and attention, and their interrelationships in psychiatric disorders, primarily schizophrenia. The overall strategy involves studies of ANS and attentional relationships to diagnosis and prognosis, studies of the effects of drugs and other therapeutic interventions, "high risk" and personality studies in normal volunteers, and studies of the measurement of ANS activity.

B. Methods Employed

The general methods of these studies include measurement of ANS activity through skin conductance (SC) usually measured bilaterally, heart rate (HR), vascular activity (skin temperature and finger pulse volume), and respiration while subjects are resting, exposed to a series of nonsignal tones of constant or of variable intensity and performing tasks. Tasks include tests of attention using reaction time techniques, tests of perceptual speed using two-flash discrimination and tachistoscopic recognition, and tasks designed to be moderately stressful. A mini-computer system is used to run the experiments and to collect and analyze the data. Studies in various stages of completion are listed below.

1. Schizophrenia Studies

a. A study of newly admitted, drug-free patients used a "balloon stress" (blowing up a balloon until it pops) and two tests of perceptual speed given on different days. The tachistoscope task allows testing of the hypothesis, developed in previous studies, that schizophrenics' ANS does not respond appropriately to variations in stimulus significance. This study also includes several rest periods and a series of nonsignal tones for comparative purposes.

b. In current studies, ANS recording is being carried out in two sessions of rest, tone series, and reaction time. In addition, several methods of

assessing attention deficits using reaction time (RT) techniques are being compared: (1) the classical "set" procedure of Shakow which involves variations in the foreperiod in a simple auditory RT paradigm, (2) RT to visual and auditory stimuli, measured when the stimuli are predictable, unpredictable, or simultaneous (but unpredictably so). We have confirmed previous findings in normals that although RT to tones is faster than RT to lights when the stimuli are predictable, RT to light is faster under unpredictable simultaneous presentation. This is taken to indicate an attentional bias toward visual stimuli or visual dominance, (3) comparison of ipsimodal vs. crossmodal sequences of tones and lights in a simple RT paradigm, plus occasional simultaneous presentation to assess "intersensory facilitation." Simple RT is faster under simultaneous presentation of a tone and light in the context of an unpredictable series presumably because the subject's response is triggered by whichever of the two stimuli he is attending to.

c. Patients are being tested during their hospitalization using a protocol of rest periods, a series of variable intensity tones (60-100 dB), and a two-flash discrimination procedure. Patients in this study are on an active treatment or placebo. Drugs, such as pimozide, lithium, naltrexone, GHB, and propranolol, and prazosine and other treatments, such as hemodialysis and plasmapheresis are evaluated.

2. Studies on Nonschizophrenic Psychopathology

a. Patients with depressive and obsessive compulsive disorders have been tested shortly after hospital admission or as outpatients (on a protocol identical to the first part of the schizophrenics' protocol described in l.b. above) in collaboration with the CNB. Patients were tested while being treated with the tricyclic antidepressant clomipramine and the Type A monoamine oxidase inhibitor clorgyline as in l.c. above. Adolescent obsessive-compulsive patients and aged-matched controls are also being studied in collaboration with LCS.

b. In collaboration with BPB, ten cases of multiple personality have been tested in 4-5 sessions each on short versions of the rest, tones, and RT time procedure. The method is to test the same three different personalities in a different order in each session to control for adaptation and compare the between-personality variance to the within-personality variance. Normal controls are now being tested.

c. A study of women in different phases of their menstrual cycle has been initiated in collaboration with CNB and BPB. Women who report varying degrees of premenstrual discomfort, ranging from none to clinically diagnosable affective disorders are being studied. In addition to providing information on ANS involvement in premenstrual tension, we hope this study may help elucidate state and trait issues in ANS functioning in psychopathological conditions.

d. Men who had a diagnosis of early infantile autism are being tested with part of our standard protocol in collaboration with LCS.

e. Patients with anxiety and panic disorders are being tested at baseline in collaboration with BPB. Further tests during drug protocols are planned. High ANS activity has been closely associated with anxiety disorders. This project may determine if variations in ANS activity are associated with clinical differentiations in this group of disorders and with response to different treatments. In addition this project is relevant to the questions of the specificity of ANS markers to particular disorders and the biological determinants of ANS activity.

3. Studies on Normals

a. Two "high-risk" studies in which subjects were selected on the basis of performance on attention tasks, have been carried out. In one, subjects were selected for very good or very poor performance on the Continuous Performance Task, and in the other, pendulum eye-tracking was used. The procedures we have used were similar to those used in the current studies on schizophrenia.

b. Two studies of reactions to physical and psychological stress have been done in collaboration with LCS and BPB. In addition to ANS measures, measurement of changes in norepinephrine, B-endorphin, and cortisol from plasma have been made.

c. In collaboration with LSES (Project #Z01 MH 00674 LSES), a method of confirmatory factor analysis is being used on ANS and personality data from 95 normal subjects. This has the objective of developing error-free measurement models of the structures of these systems, in order to reduce the large number of variables generated by the ANS and personality assessment procedures to many fewer and more "pure" concepts. This method may lead to causal models of the interrelationships between systems.

4. Literature Review

Much of the last year has been spent in writing a literature review of the psychophysiology of psychopathology which is nearing completion. The review covers research using all psychophysiological techniques on the major psychoses, psychopathy, hyperactivity, autism, and neurotic disorders. This review should provide a framework for interpreting the data generated by this project.

C. Major Findings

1. Schizophrenic Studies

a. In a completed study, we showed that a pattern of ANS and attentional functioning - high ANS "arousal," small ANS responses, particularly to meaningful or demanding stimuli and situations, slow adaptation and habituation, and poor attention - characterized unmedicated acute schizophrenics compared to normal controls and within the schizophrenic group, predicted a poor clinical outcome of a 4-month hospitalization. Results from the newer study generally confirm those from the previous study for the schizophrenics as a whole compared to controls.

We are starting to analyze the relationships between the ANS data and symptoms, clinical course, and biologic markers. The presence or absence of Schneiderian "first rank" symptoms did not affect the ANS results in a major way, but we found that 8 patients with large sulci as measured from CT scans had significantly smaller ANS reactions to stimuli and tasks than 20 with normal CT scans. Patients without first rank symptoms who also had normal CT scans seem minimally deviant from controls on ANS measures. A negative finding is that ANS measures obtained in drug-free periods do not predict the psychological response to an infusion of amphetamine in schizophrenics.

b. Data are still being collected, but it is apparent that the phenomena of visual sensory dominance and intersensory facilitation found in normal subjects also occurs in schizophrenics.

2. Studies on nonschizophrenic psychopathology

a. When drug-free, neither the obsessive adults nor children have shown, as a group, the labile and "aroused" ANS recordings that one would expect from a disorder that has a high anxiety component. Preliminary comparison of the adolescent patients with their controls has shown a reduced ANS response to task performance. Individual differences in ANS activity will be correlated with other biologic variables and with treatment response in both the adult obsessive and depressed patients.

Clinically, clomipramine was quite effective in reducing obsessional symptoms while clorgyline had minimal effects in most patients (see Project Z01 MH 00336-04 CN). Psychophysiologically, the two drugs had rather similar effects in reducing electrodermal base levels, increasing HR and decreasing HR variability.

However, compared to both placebo and clorgyline, clomipramine significantly reduced both tonic and phasic ANS reactions to simple tones (especially those of high intensity) and to the mild stress of the two-flash discrimination task. Subjects with a better clinical response tended to show greater drug effects on ANS reactivity. The results are compatible with the hypothesis that attenuation of ANS reactions to feared situations allows obsessive behavior to be extinguished.

b. Multiple personality subjects are being evaluated on a case-by-case basis. Preliminary analyses of the data suggest consistent differences in RT among personalities in a majority of the subjects and that habituation of the skin conductance orienting response in one personality may be unaffected by the prior experience of another personality.

c., d., & e. No reportable findings as yet.

3. Studies on Normals

a. Poor attenders, as defined by the CPT, were found to have increasingly impaired reaction time as the amount of processing required increased from simple

(20-25 msec) to choice reaction time (100 msec). This deficit seems due to a problem of shifting attention - from longer to shorter foreperiods in the Shakov procedure or between stimulus modalities in the other procedures. Thus, we have found a rather specific attention deficit in normal subjects that appears on several tests and is similar in kind to what has been found in psychopathology. Subjects with poor eye tracking were not greatly impaired in choice RT but were especially slow in responding when the timing of stimulus onset was uncertain. Thus, the CPT and eye-tracking tasks seem to be related to distinct kinds of attention impairments.

b. In both stress studies, it has been found that plasma norepinephrine increases markedly to physical stress but minimally to psychological stress. Other purported indices of ANS activity, notably skin conductance and heart rate, change markedly to both types of stress. This suggests relatively greater peripheral control of norepinephrine and relatively greater central control over the other measures. Coronary prone (or "Type A") middle-aged men surprisingly evidenced higher base levels and smaller increases in norepinephrine and heart rate to physical (postural change from supine to standing) than Type B men.

c. Measurement models have been obtained successfully for concepts of skin conductance tonic arousal, skin conductance lability, skin conductance response speed and heart rate arousal for males and females in two different testing sessions, and for concepts of activity and mental health derived from a large number of personal history and personality variables. The strongest relationships found indicate that for women activity is negatively correlated with cardiovascular activity and for both genders perceptual speed is positively correlated with skin LPP conductance indices of ANS arousal. Platelet monoamine oxidase activity correlates negatively with active behavior and positively with skin conductance activity.

Significance to Biomedical Research and the Program of the Institute

Investigations of ANS activity and attention in psychiatric disorders, especially schizophrenia, have produced promising results which suggest that these processes may play fundamental roles in the etiology and expression of the disorders. Limitations on inferences to be drawn from measures of ANS activity come from incomplete understanding of their biological and psychological determinants. One of the main goals of this research is to increase this understanding by investigations of biological and psychological correlates and improving measurement techniques. The dynamic nature of these measures permits the study of processes, such as adaptation, habituation, response to and recovery from stress, and effects of single stimuli through noninvasive techniques. Thus, further understanding of their mechanisms could greatly increase their utility in investigations of psychopathology. Continued investigations of the diagnostic specificity of these processes and of their relationships to other clinical features and to prognosis are necessary to confirm and extend our previous results and to test the limits of their generality.

Proposed Course

Analysis will continue of data for the completed project on schizophrenia with the goals of determining the relationship of ANS variables to diagnosis, diagnostic subtype symptomatology, severity of psychosis, performance on tests of attention and perceptual speed, degree of improvement during hospitalization and improvement on specific treatments. ANS activity in patient groups will be studied in relation to data obtained from biochemical assays of body fluids such as monoamines and their metabolites in CSF and monoamine oxidase activity.

Collection of data will continue for current projects on schizophrenic and nonschizophrenic psychopathology. Normal controls will be tested on the same protocol. This protocol will be used in the collaborative LPP project on attention disorders.

Investigation of ANS and behavioral effects of various pharmacological therapeutic agents will continue for all these groups with the purposes of determining the comparative effects of the drugs and correlates with clinical response.

Data analysis will be completed on the high risk projects for group comparisons and correlation with other data on the same subjects. We will complete the general model of ANS task performance, personality, and mood variables to attempt to model other features of the data such as adaptation and habituation. If these methods prove useful, we will apply them to other data bases for both patients and normal subjects.

Publications

Zahn, T.P.: Autonomic nervous system markers of diagnosis and prognosis in schizophrenia. In Hanin, I. and Usdin, E. (Eds.): Biological Markers in Psychiatry and Neurology. London, Pergamon Press, 1982, pp. 347-358.

Bernstein, A.S., Frith, C.D. & Zahn, T.P., Gruzeliier, J.H., Patterson, T., Straube, E., Frith, D., and Venables, P.H.: An analysis of skin conductance orienting response in samples of British, American, and German schizophrenics. Biol. Psychol. 14: 185-211, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00486-11 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Psychophysiological Concomitants of Minimal Brain Dysfunction in Children</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Theodore P. Zahn, Ph.D., Research Psychologist LPP/DCBR/IRP/NIMH</u>		
COOPERATING UNITS (if any) <u>Laboratory of Clinical Science, NIMH</u>		
LAB/BRANCH <u>Laboratory of Psychology and Psychopathology</u>		
SECTION 		
INSTITUTE AND LOCATION <u>NIMH/ADAMHA, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <div style="text-align: center;">0.3</div>	PROFESSIONAL: <div style="text-align: center;">0.2</div>	OTHER: <div style="text-align: center;">0.1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Many investigators believe that the <u>autonomic nervous system</u> (ANS) may be involved in <u>hyperactivity</u> (HA) in <u>children</u>. The objectives of our studies are to investigate differences in autonomic functioning between HA and normal children by means of peripheral indicators, such as <u>skin conductance</u>, <u>heart rate</u>, and <u>skin temperature</u>, and to assess the effects of the drugs on both autonomic activity and on task performance which depends on <u>attention</u> such as <u>reaction time</u>. The test protocol includes recording the ANS variables during a rest period, presentation of a series of simple tones, and a reaction time task. The hypothesis that HA and normal children respond differently to stimulant drugs has been tested in a study of the effects of <u>d-amphetamine</u> on autonomic activity and attention in 6 to 13-year-old normal and HA boys. On the same protocol, male adults have been tested for age differences in drug effects. Studies have been done on the acute and chronic effects of <u>caffeine</u> on these measures in boys and in normal men. Results show that caffeine increases ANS activity in a manner similar to anxiety and that there are differences in placebo ANS activity and perhaps in caffeine effects among subjects according to their habitual caffeine usage. A new study of chronic (2-week) periods of caffeine or placebo intake is being carried out on children using an experimental design that should control for acute withdrawal effects.</p> <p>These studies are designed to determine the role of autonomic activity in behavioral effects of stimulant drugs.</p>		

Other Professional Personnel

Judith Rapoport, M.D., LCS, NIMH

Project Description

The general purpose of this project is to investigate the role of autonomic nervous system (ANS) activity and attention in hyperactivity in children and to study the effects of stimulant drugs on these processes in hyperactive and normal children and in normal adults.

Studies have been finished testing the effects of caffeine in boys and men using the same experimental procedures as were used in the amphetamine studies. The objectives of these studies are to compare the effects of these two "stimulant" drugs and, since caffeine has been reported to be a competitive inhibitor of the benzodiazepine receptor, to evaluate it as a possible pharmacological model of anxiety.

Results of these studies have been detailed in previous annual reports and the amphetamine studies have been published. Papers focusing on the attentional and ANS effects of caffeine in children and adults are in the planning stage. A new study on chronic caffeine use in children is being carried out with Dr. Rapoport (See Project #Z01 MH 00161-05 LCS) to correct some of the methodological difficulties in the previous one in which the effects of chronic caffeine consumption, acute withdrawal from caffeine in habitual users and personality traits leading to caffeine consumption were confounded. In the new study, subjects are "on" or "off" caffeine for two weeks which should be long enough to separate these factors.

Significance to Biomedical Research and the Program of the Institute

These studies are significant for biomedical research and the program of the Institute in several ways. First, the study of ANS changes after drug administration may help elucidate the mode of action of the clinical effects. Second, since the pharmacological effects of these drugs are partially understood, these studies can elucidate the mechanisms of ANS activity and help to interpret the ANS findings on clinical populations. Third, amphetamine abuse is a public health problem and caffeine abuse may be one, especially in children. Further understanding of the biological and psychological effects of these drugs may help in dealing with these problems.

Proposed Course

In addition to the new study on chronic caffeine use in children described above, the future course of the project will include a detailed examination of the nature of the attention deficit in hyperactivity (now called "Attention Deficit Disorder" in DSM III) in line with the general program of this laboratory to develop a taxonomy of attention disorders.

Publications

None. (See #Z01 MH 00161-05 LCS)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00491-07 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Personality Factors and Psychophysiological Responses to Changing Stimulus Input

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Theodore P. Zahn, Ph.D., Research Psychologist, LPP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.5

OTHER:

0.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The objectives of this project are to investigate relationships among differences in personality, sensory thresholds, and autonomic nervous system (ANS) activity in normal humans and to study racial differences in ANS activity. Bilateral skin conductance and heart rate have been recorded in two sessions in which constant and variable intensity tones and lights are presented and auditory and two flash thresholds determined by methods which permit signal detection analyses. Several standardized personality tests were also given. These include scales of sensation seeking, extraversion, neuroticism, psychoticism, field dependence and anxiety. In addition comprehensive measures of lateral dominance have been given as well as a measure of "torque" (clockwise drawing of circles) which is thought to reflect a neurointegrative deficit and be related to risk for future psychopathology. The procedures allow determination of the effects of stimulus intensity and heteromodal stimulation on ANS activity. A procedure for manipulating ANS arousal experimentally with minimal distracting effects--a change in posture from supine to standing--is being used to assess the effects of arousal on performance and the effects of personality variables on this relationship. This project allows testing of several theoretical models of the relationships of ANS activity, sensory sensitivity, and personality, some of which have implications for the etiology of psychopathology. Tests of the relationships between laterality in skin conductance variables and behavioral laterality will also be done to see if inferences about lateralized brain function can be made from such variables.

Project Description

A. Objectives

A large body of psychological literature postulates that an important dimension of individual differences in behavior or personality is reflected in the reactions of the nervous system to sensory stimulation. Pavlov's original conception of "strong" and "weak" nervous types has been modified and extended by Western theorists to reflect such personality dimensions as "extraversion-introversion," "sensation-seeking," and "field dependence," each of which can be measured by a questionnaire or other test procedures. The theoretical models that have been built up from these concepts have implications for interrelationships among personality, autonomic nervous system (ANS) base levels and responsivity to stimulation, and sensory sensitivity. There are also implications for psychopathology in that schizophrenics have been considered to be extremely "weak" nervous types in the Pavlovian system (i.e., overreactive to weak stimulation and underreactive to strong stimulation--"transmarginal inhibition"). Another development is the more recent delineation by H. Eysenck of the dimension of "psychoticism."

The major objective of this project is to test some of the implications of these models of personality by interrelating the personality measures with sensory thresholds and sensitivity, and ANS activity in normal humans. Other objectives are to assess racial differences in ANS activity and in its relationships to the other variables in the study and to explore relationships of differences in the laterality of skin conductance activity with behavioral assessments of laterality, and to test the effects on ANS activity increasing arousal by means of a postural change.

B. Methods Employed

Over 180 normal volunteers have been assessed on several personality dimensions, including the Eysenck scale of extraversion, neuroticism, and psychoticism, field dependence, sensation-seeking, impulsivity, ego strength, and anxiety, assessed for degree of lateral dominance, and given tests of ANS and sensory functioning in two separate sessions as described earlier.

In a second protocol, the effects of changes in posture on ANS activity during rest, a series of 86dB tones and a TFT task is assessed. Subjects are tested when they are reclining or standing on two separate days in counterbalanced order. A total of 47 subjects have been tested on these procedures.

C. Major Findings

Analyses of data from 145 male subjects given the first protocol have confirmed the preliminary findings that subjects scoring high on extraversion manifest generally low ANS activity under virtually all conditions. Surprisingly, the group showing the highest ANS activity were those in the middle range, referred to as ambiverts. Another surprising result was that subjects scoring

high on a neuroticism scale were significantly lower in ANS activity than those with low neuroticism. Further, there were interactions between these two personality dimensions such that ambiverts with low neuroticism showed evidence of greater responsivity to tones, but neurotic introverts showed the largest dishabituation effects.

In terms of relationships to psychopathology, extraversion has been associated with psychopathy, delinquency and hyperactivity and neuroticism with anxiety states. Since ANS reactivity to stimuli has been shown to be low in all these conditions, the data are consistent with the notion of a continuum between normal personality variations and the psychopathological conditions. Evidence of low ANS baseline activity in high neuroticism subjects does not fit with the findings from their clinical counterparts, however. This suggests that high baseline activity may be a state-related phenomenon which emerges only with overt psychopathology.

The effect of a postural change from supine to standing was, as expected, to increase base levels of electrodermal and heart rate indices of arousal. In addition, the frequency and amplitude of skin conductance orienting responses to tones were also increased as were the amplitudes of the heart rate responses. Thus the postural change was successful in producing a change in ANS baseline arousal, and the change in responsivity was what might be expected from previous studies of between-subject correlations of baseline activity and responsivity.

The effects of the postural changes, in preliminary analyses, seem to depend on the personality dimension of psychoticism in a manner consistent with previous between-subject findings, but more subjects will be needed to confirm this.

Subjects exhibiting a "torque" pattern of drawing a circle (clockwise motion), a pattern thought to reflect a neurointegrative deficit and increased risk for future psychosis, had impaired sensory sensitivity and low ANS baselines compared to nontorque subjects.

Significance to Biomedical Research and the Program of the Institute

Further understanding of how autonomic, perceptual, and personality variables interact in normal subjects should be of great assistance in interpreting the autonomic and perceptual results from studies on psychopathology in which similar methods are used in our other studies. Similarly, the study of racial differences in normals will help us evaluate the results of racially mixed samples of patients. This project has been very useful in the development of protocols for studies of psychopathology.

Proposed Course

The sample size of the postural change study will be increased to permit testing of the effects of individual differences in personality. Analysis of the older data will continue with special emphasis on lateral differences in electrodermal activity. There is much confusion in the literature about the interpretation of such differences, but there are some interesting findings in

psychiatric patients. Since this is one of the few studies in the literature to test a large sample of left-handed subjects, the data should be of value in such interpretation.

Publications

None

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00495-07 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Psychobiology of Cognitive Processes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Herbert Weingartner, Ph.D. Chief, Unit on Cognitive Studies LPP NIMH

COOPERATING UNITS (if any) Biological Psychiatry Branch, Lab. of Clinical Science, Clinical Neuropharmacology Branch, Clinical Psychobiology Branch, NIMH; NIAAA; NIDA; Gerontology Branch, NIA (NIH); NINCDS (NIH); Walter Reed Hospital; Veterans Administration; Department of the Army and Navy

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

2.0

PROFESSIONAL:

1.8

OTHER:

0.2

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The aim of these research efforts is to explore the psychobiology of cognition in man. We attempt to interrelate psychological and biological determinants of the various components of cognition. Studies have been designed to understand the specific and discrete psychobiological mechanisms that define the acquisition, processing, encoding, consolidation, and retrieval of experience. Other studies have also begun to examine the meta-cognitive processes involved in learning and memory. Experiments are also designed to examine the biological and psychological determinants of psychiatric and neuropsychiatric alterations in cognitive processes in adults and children. Specific forms of central nervous system dysfunctions (e.g., as defined by type of lesion in neuropsychiatric disorders) may affect specific and distinct components of cognitive processing. Similarly, psychoactive drugs that affect discrete aggregates of neurons may alter different aspects of cognition and information processing. Based on empirical studies of clinical populations (e.g., depression, Alzheimer's disease, Korsakoff's disease, forms of learning impairments in children) and several types of psychoactive agents (cholinergic drugs, noradrenergic drugs, neuropeptides), it has been possible to begin to define the psychobiological relationships between semantic and episodic memory, encoding processes, and effortful (active) cognitive operations as opposed to automatic cognitive processes.

Other Professional Personnel

Robert Cohen, M.D., LPP, NIMH
 Walter Kaye, M.D., LPP, NIMH
 Barbara Strupp, Ph.D., CPB, NIMH
 Frederick K. Goodwin, M.D., DIRP, NIMH
 Dennis L. Murphy, M.D., Chief, CNB, NIMH
 Barbara Perry, M.D., LCS, NIMH
 Thomas Wehr, M.D., LCS, NIMH
 Richard Ross, M.D., LCS, NIMH
 Marku Linnoila, M.D., NCAAA, ADAMHA
 Philip Gold, M.D., BPB, NIMH
 Stanley Burns, M.D., LCS, NIMH
 Richard Newman, M.D., NINCDS, NIH
 Jordan Grafman, Ph.D., Department of Clinical Investigation,
 Walter Reed Army Medical Center
 Stanley Rapoport, M.D., NIA, Gerontology Research Center

Project Description

The research projects reviewed here are all concerned with the psychobiology of cognitive processes. They have been designed to explore the psychological and biological determinants of various aspects of cognitive processes and their interrelationships. Studies have examined the psychobiological processes that define the encoding, processing, learning, and storage of information, how processed events are altered, or elaborated in memory, the consolidation and retention of information, and the mechanisms that are involved in retrieval of stored information. Other research efforts have also begun to examine how we know what is and is not in memory. Some of the components of cognitive processes that have been examined include attentional determinants, aspects of short-term memory, the consolidation of information, long-term memory, state and trait specific cognitive strategies, effort demanding vs. automatic cognitive processes, and the kinds of strategies that subjects use to retrieve experience that they have stored in memory. Recent studies have also focused on the distinction between the psychological and biological determinants of episodic and semantic (knowledge) memory as well as meta-cognitive processes associated with information processing.

Two broadly defined types of strategies are used to define the psychobiology of cognitive processes. One is to contrast the effects of different treatments on different components of cognition. These studies include: (1) pharmacological manipulations, such as cholinergic drugs, noradrenergic drugs, abused drugs (alcohol, marijuana), central nervous system depressants, neuropeptides, and (2) behavioral manipulations that alter reinforcing properties of stimuli arousal/activation, stimulus attributes (altering encodability), and types of stimulus processing strategies subjects use to process information. These studies are carried out in unimpaired subjects, as well as in patient groups with different forms of psychopathology such as disorders of mood, dementing disorders and patients with localized brain injuries. Would different kinds of pharmacological or behavioral manipulations of cognition lead to different forms of

enhanced or disrupted cognition? Contrasting different treatment effects on different CNS systems and relating these changes to cognitive responses should be particularly useful in providing us with a picture of the structure of the psychobiology of cognition. A second type of strategy for researching the psychobiology of cognition is to contrast different forms of cognitive failures as seen in different psychiatric and neurological syndromes. Would disruptions in cognition seen in some psychopathological states be qualitatively and quantitatively unique and related to specific changes in central nervous system activity? For example, what are the differences in the amnesic and cognitive impairments seen in Huntington's disease, Korsakoff's syndrome, and Alzheimer's disorder? To what extent are cognitive changes associated with aging or the "pseudodementia" evident in some Parkinson's Disease patients different from impairments evident in dementing disorders? How might the differences be an expression of the specificity of central nervous system involvement in each of these disorders? In some instances, the possibility of discriminating between the form of the cognitive impairment is necessary for both adequate diagnosis and effective treatment, i.e., such as the cognitive disruptions that are part of depression as opposed to that seen in a progressive dementia of an Alzheimer's type. Frequently, depression is an integral part of a progressive dementia, and the cognitive impairment is a joint product of the two disorders. A number of studies have been designed to investigate the therapeutic potential of various psychoactive drugs and behavioral treatments. These studies focus on whether such treatments attenuate or reverse the cognitive disruptions seen in various forms of dementia, hyperactivity, and learning disability syndromes in children, depression, mania, and the schizophrenias.

In summary, each of the studies is clinically relevant while serving as a basis for understanding the underlying psychobiological determinants of cognitive processes. To accomplish this each project is concerned with some aspect of the problem that is associated with defining the discrete psychobiological components of cognitive processes that are involved in the appreciation, storage, retention, and retrieval of experience. The studies are concerned with the determinants of discrete cognitive processes as well as clinical studies of disordered cognition.

1. Semantic (Knowledge) Memory and its Relationship to Other Forms of Learning and Memory (Episodic Memory)

Studies have been designed that would begin to describe how knowledge is represented in memory (semantic memory) and how it would be accessed and used in order to encode or appreciate ongoing events. The relationship between semantic memory and episodic memory, how these are altered by various biological treatments and how these processes break down in neuropsychiatric disorders represents a very new and important area of investigation in exploring the psychobiology of cognition. It is also a particularly important area of study in order to understand the cognitive failures evident in progressive dementias such as of an Alzheimer's type.

2. Pharmacological Alterations (Enhancement and Disruption) of Cognitive Processes

These studies have contrasted the cognitive enhancing effects of drugs which differ in terms of their effects on central nervous system activity. Neuropharmacological strategies of particular interest are serotonergic agents (Zimelidine); dopaminergic drugs (L-dopa/carbidopa); cholinergic agents (such as arecoline, physostigmine, THA, and lecithin treatment); amphetamine; various forms of drugs that alter neuropeptide activity (vasopressin, naloxone) in both unimpaired subjects, as well as in patient groups (hyperactive children; patients suffering from various forms of dementia such as Alzheimer's and Huntington's disease, Korsakoff's disease, aging and in depressed patients). Do drug treatments that affect different aggregates of neurons in the central nervous system produce different kinds of changes in cognitive processing? Are enhancements or disruptions of cognition determined through different psychobiological mechanisms, and might the cognitive response to different drugs make such a pattern discernible? Might some neurotransmitter antagonists also mimic or model forms of syndrome specific disorders of cognition in man? In some instances, drugs that might disrupt aspects of the acquisition of information may enhance some other stage of cognitive processing (e.g., consolidation). Neuropharmacological tools that, on the one hand, model forms of disturbed cognition, or strategies that prove useful in treating cognitive dysfunctions can also be used to better define a psychobiology of information processing in man. This research has also involved attempts to find clinically useful drug treatment strategies for altering disrupted cognition in man.

3. State-Dependent Learning

This area of research has provided a useful framework for exploring: a) the qualitatively unique manner in which events are stored (encoded and later retrieved from memory); b) how multiple personality configurations can serve as context markers in the encoding and retrieval of information; c) studies of disturbances in mood state and how these define mood-specific strategies for processing experience and remembering past events in memory; d) qualitative changes in cognition in response to psychoactive drugs; e) contextual factors as determinants for defining the nature of trace events in memory; and f) individual differences in susceptibility to state-dependent or dissociative mood/drug effects.

4. Memory Consolidation

This research has focused on the psychobiological events that follow the acquisition (storage) of information and the events that occur before processed information is to be retrieved from memory. Studies in both normal subjects and patients have examined the form and strength of stored information in memory and the processes that might further sustain, enhance, or disrupt stored trace events that are already part of memory. Information in memory is altered in time, including changed in form. The experience of knowing that some event occurred earlier also changes as memories are transformed during a consolidation phase of

information processing. The psychobiological events that play some role in the rate of decay of information in memory and the susceptibility of information to interference may be important determinants in defining what is available and accessible in recall, once information has been stored in memory. Disruptions in consolidation may contribute to the cognitive failures in the dementias as well as in amnesic syndromes.

Drugs that disrupt memory and learning may do so by altering biological operations that succeed acquisition or learning. Likewise, drugs (e.g., neuropeptides) may enhance aspects of learning and memory by facilitating the consolidation of learned information.

5. Behaviorally-Defined Mechanisms that Alter Components of Cognition

Characteristics of stimuli such as: a) organizational and relational properties; b) imagery inducing properties; c) emotional arousing attributes of stimuli; d) information presentation rate; e) mode of processing; f) acquisition of language vs. pattern information; and g) kinds of learning such as automatic vs. effort demanding cognitive operations can all change the characteristics of learning and memory. These factors have each been studied in relation to its effects on attention, acquisition (learning), strength of learning, retention, and components of information retrieval. The studies have examined these determinants in normal controls, as well as in patient groups (depressed patients, hyperactive children, learning disabled children and patients suffering from various forms of dementia). Some of the issues raised in these studies include the following: How might aspects of information processing alter the attentional, short-term memory, encodability, retention, and retrieval of information? Would different forms of psychological manipulations systematically alter different aspects or components of cognitive processes? Do patients who demonstrate failures in learning and remembering do so because of disruptions in some, but not all of these component cognitive processes, and can manipulations of some characteristics of information processing change or attenuate these disruptions in cognition?

Recent studies have also begun to explore the relationship between the reward-reinforcement systems along with "effortful" vs. "automatic" information processing and how these are altered under different motivation/arousal conditions. This approach to cognition has been particularly useful in defining the cognitive changes in depression, the determinants of cognitive failures in the learning-disabled child, pseudementia in contrast to cognitive impairments in progressive dementias of an Alzheimer's type and drug-altered changes in cognition (see below).

6. Cognition and Mood

Studies have included research of mood-related changes in: a) depression in relation to brain lateralization of cognitive functions; b) arousal and activation and its role in information processing; and c) the encoding and retrieval of events in unimpaired subjects where mood also can be variable as well as in

disordered mood in depression and mania. This research has examined how patients with disturbances in mood process information in a mood-state specific manner. In addition, this research has begun to examine mood-related changes following psychoactive drug treatment and its interactive role in altering cognitive processes. Other research has explored the degree to which effortful processing of information is compromised as a motivation-related determinant of thinking in depressed patients.

7. Mechanisms of Cognitive Impairments that Determine Forms of Learning Disabilities

Studies have been designed which investigate: a) forms and incidence of various kinds of learning disabilities in children; b) the nature of the learning disabilities in these children; and c) potential strategies for their remediation.

Methods

Three strategies have been used in these studies. One involves manipulation of different biological systems that may play a role in different aspects of cognition in man. Various neurotransmitter agonists and antagonists, as well as agents that affect neuroendocrine functioning are contrasted in both impaired and unimpaired subjects. A second strategy involves systematic comparison of various forms of cognitive failures apparent in different clinical groups. Methods used include measures and assays for evaluating neuropathological, neurochemical, and neuroanatomical changes that are apparent in different clinical syndromes. These data provide a matrix for relating biological variables with measures of different components of cognition as seen in forms of impaired cognition. The third set of methods involves systematic manipulation of acquisition conditions, stimuli, retention processing, and retrieval conditions.

Several types of cognitive procedures and strategies have been designed to explore the psychobiological components of cognition in the studies that are part of this project. Many of these methods are modified forms of current techniques used in human information processing research, as well as newly-developed tools that might more adequately examine determinants of cognition in clinical studies and those assessing cognitive drug effects. These strategies have been developed in a number of studies and include measures and manipulations of: a) organization of information; b) informational context for processing information; c) stimulus attributes such as imagery, emotional properties and frequency; d) forms of learning and recall (free recall, prompted free recall, recognition memory, cued recall, serial learning and paired associates learning); e) processing time and type of presentation of information; f) type of processing strategy (processing on the basis of meaning or sound properties); g) immediate vs. delayed recall of information with or without rehearsal of stored information; h) presentation of language vs. pattern information to left vs. right hemisphere (methods used to investigate lateralization); i) rapid (tachistoscopic) presentation of information; j) measurement and manipulation of different forms of retrieval of information in memory, including forms of free recall, prompted or cued recall, recognition memory, method of "savings"; k) very

long-term memory retrieval; l) assessment of effortful and automatic cognitive processes; and m) arousal and motivation in information processing.

Most recently new cognitive methods have been developed which also permit us to examine characteristics of semantic (knowledge) memory in contrast to the methods described above which are primarily useful for describing episodic memory. These methods allow us to measure the structure of knowledge in memory and how readily it can be accessed and used in transforming events. This is being accomplished through the development of new behavioral techniques as well as psychophysiological and neurobiological methods (positron emission and versions of event related and average evoked response methods).

Findings: Psychobiology of Cognition

1. Semantic Memory, Episodic Memory, Automatic and Effortful Cognitive Processes: Psychobiological Relationships

A series of studies has shown that semantic memory (knowledge memory) and episodic memory are psychobiologically distinct but interrelated types of information processing systems. We have shown that a) failure to access semantic or knowledge structures in memory is the major cognitive impairment that is the determinant of the dementia in progressive neurological disorders such as in Alzheimer's disease; b) that episodic memory failures are determined by semantic memory impairments in this type of progressive dementia; c) other amnesic

syndromes (such as in Korsakoff's disease) are due to different psychobiological determinants, other than those that affect access to semantic memory; and d) the cognitive disturbance in depression is due to a specific disruption in effort demanding cognitive operations while automatic processes and semantic memory functions are left relatively unaffected - this same pattern of cognitive impairment is also evident in early stage Parkinson's Disease. These findings have been useful not only because they elucidate basic mechanisms of learning, memory and related cognitive processes but they provide new and more effective diagnostic tools for distinguishing types of cognitive dysfunctions.

Related neuropharmacological findings include: a) neuropeptide treatments such as arginine vasopressin facilitate access to semantic memory; b) serotonergic drugs, such as Zimelidine appear to enhance memory by amplifying weak, poorly processed memory traces; c) L-dopa treatment appears to produce a specific enhancement of effort demanding cognitive operations implicating the dopamine system in this type of memory-learning function.

2. Cognitive Impairment in Progressive Dementia, "Pseudodementia" and Korsakoff's Disease and Possible Treatment Strategies

Recent findings from our laboratory have defined some of the characteristics and determinants of the cognitive dysfunction in progressive dementia patients. We know that information is relatively rapidly lost from memory; immediate memory is relatively unimpaired, and any type of learning-memory operation that requires

the establishment of permanent trace events in memory is dramatically disrupted. Memory failures are, in large part, due to processing or acquisition deficits which then result in weak trace formation and therefore failures to retain information in memory. A considerable body of research has suggested a distinction between semantic memory and the repository of information of knowledge structures from episodic memory, i.e., memory for ongoing recent events. Although these two kinds of memory systems have been traditionally viewed as being separate and distinct, we have found an important link between the two. Based on recent findings relating these two systems, it has been possible to account for many aspects of the memory impairment in progressive dementia patients. In a series of studies, we have been able to demonstrate that the extent to which Alzheimer's patients have access to structures in semantic memory is the extent to which they are relatively unimpaired on many tasks of learning and memory. These results have important implications both diagnostically, in distinguishing this group of cognitively impaired patients from other groups (e.g., cognitively impaired depressed patients), as well as for the development of potential treatment strategies.

Parkinson's Disease (PD) patients also demonstrate learning-memory problems that can be quite severe. We have found these impairments to be qualitatively different from those evident in Alzheimer's disease. PD patients manifest impaired cognition on effort demanding cognitive tasks but not when information can be processed relatively automatically. Access to semantic memory is also left unaffected in the early and middle stages of the disorder. L-dopa treatment, a common drug used in PD, produces a facilitation of these same cognitive component processes in unimpaired older subjects.

Most recently we have been able to show some facilitation of learning and memory in progressive dementia patients using two very different strategies. Cholinergic drugs seem to produce small improvements in learning and memory but only in those patients that are least cognitively impaired. In contrast, arginine vasopressin enhances learning and memory by facilitating access to semantic memory (a mechanism of action that is consistent with the determinants of the memory failure in these patients).

Although Korsakoff patients (KD) are often as memory impaired as progressive dementia patients, the cognitive and biological determinants of their impairments are quite different. Unlike progressive dementia patients, the Korsakoff amnesia patient responds to attributes of stimuli that would ordinarily aid encoding such as a) repeating information, b) organizing information, and c) presenting pictures rather than words. Furthermore, the Korsakoff patient (KD) can learn procedures and remember them for very long periods of time. This is because unlike progressive dementia patients the Korsakoff patient (KD) is able to access semantic memory.

In attempting to reverse the amnesic-like impairment in KD we have tried drugs that would affect the noradrenergic system. Thus far we have been unsuccessful in producing reliable improvements in KD memory functions using clonidine as a drug strategy.

These findings, when examined together, have suggested that cognitive failures in progressive dementia are distinguishable from those evident in depression and other syndromes. This has prompted active study of drug and other treatment strategies for reversing such cognitive failures. By understanding both the mechanisms of cognitive impairments and the neurochemical response following various forms of drug treatment, it should be possible to design studies that would examine the therapeutic potential of various types of drug treatments. The mechanisms and determinants of the cognitive impairments in depression and dementia have allowed us to devise strategies that should prove useful in distinguishing between these two groups of patients. Characteristics of automatic versus effortful processing, the extent to which effort is extended in accomplishing tasks, and the processing of unrelated vs related events allows us to begin to differentially diagnose the cognitive impairment in depression from that seen in the progressive idiopathic dementia patients.

3. Cognitive Changes in Depression

The pattern and determinants of cognitive changes in depression have been shown to be distinguishable from those expressed in other disorders (particularly in early stage progressive dementia). Depressed patients demonstrate a type of disordered thinking, one that is manifest in an inability to accomplish focused, sustained analysis of information leading to impairments in concept learning, acquisition of information, and memory. This may be related to alterations in the function of cerebral lateralization involved in processing language vs non-language information.

4. Learning Disabilities in Children

Drug treatments, such as stimulants, appear to facilitate learning and memory in some types of learning disabled children. These cognitive effects are seen primarily for those processes that require sustained effort. These effects are apparent and independent of other clinical changes in these amphetamine-treated children. In addition, learning that occurs in the amphetamine-treated state does not appear dissociated when remembering takes place in the untreated state. This is not like the kinds of dissociative, state-dependent, learning and memory effects that are seen in stimulant treated adults. These results are also important in considering the effects of stimulant treatment on the educational experience of learning disabled or hyperactive children.

In a series of studies, we have attempted to describe the components of cognitive changes that are apparent in children with various forms of learning disability. We have examined two groups of these children, one where hyperactivity is part of the syndrome, and a second group where there is no evidence of hyperactivity or generalized retardation. Nevertheless, these children demonstrate dramatic impairments in learning and memory that resemble the kinds of disruptions in cognition that are evident in some groups of adults. The resemblance is closest to depressed patients; it also resembles the kinds of cognitive changes that are produced by drugs that disrupt cholinergic and noradrenergic activity. These children show impairments in effortful processing of

information; automatic processing is left relatively intact. On incidental learning paradigms, these children are indistinguishable from normal controls. Both groups of children also demonstrate impairments in those characteristics of cognition that require the imposition of organization in memory. In many ways, the results we have obtained to date would suggest that the type of cognitive impairment seen in these children resembles that seen in depressed patients in contrast to the pattern of cognitive impairments evident in progressive dementia patients. The kinds of cognitive impairments are also like those that are apparent when unimpaired subjects are treated with drugs that disrupt or block catecholamine activity.

5. Meta-cognitive Processes in Amnesic Syndromes and Schizophrenia

Recently completed studies have demonstrated that unimpaired subjects are sensitive to many aspects of their own processing operations and memory for previously acquired events. Unimpaired subjects "know" how well something has been learned and the likelihood that something remembered is likely to have occurred or to have been part of the memory reconstruction processes involved in remembering events. Seriously impaired progressive dementia patients, despite their memory failures, can accurately judge whether an event was part of their reconstruction of their memory for previously processed events as compared to recall of some trace of that event. Korsakoff psychosis patients, who are similarly memory impaired, cannot accurately judge characteristics of their own memory. They demonstrate a dissociation or delinking between limbic (old brain) systems and the processing associated with neo-cortical areas. In recent experiments we have also noted this same type of cognitive dysfunction in schizophrenia patients.

6. Neuropharmacological Studies of Cognition in Man

We have been able to demonstrate, both in patient groups as well as in unimpaired subjects, that the effects of cholinergic antagonists and agonists produce cognitive changes that are qualitatively different from those of drugs that have their major effect on catecholamine activity. There appears to be further specificity and distinctiveness in the role of neuropeptides such as synthetic vasopressin-like substances, and of naloxone, in determining aspects of learning and memory in both cognitively impaired patients (depressed patients, alcoholic Korsakoff amnesic syndrome patients and progressive dementia patients) as well as in unimpaired subjects. Different neurotransmitter systems and different kinds of neurochemical mediators are involved in the regulation of various aspects of episodic memory (acquisition, retention, and retrieval of information) while other biological determinants appear to influence semantic memory processes. Furthermore, effortful cognitive operations appear to be determined by different biological mechanisms than those involved in automatic cognitive operations.

We have demonstrated that cholinergic mechanisms play a role in aspects of information acquisition and in the storage and retrieval of information. Scopolamine treatment, which disrupts cholinergic activity, produces an

impairment in information processing. This scopolamine-induced impairment in the acquisition of new learning can be reversed by arecoline treatment. The scopolamine induced disruption in cognition appears to model, in normal subjects, many of the characteristics of cognition seen in untreated progressive dementia patients.

In another study, it was possible to show that cholinergic mechanisms may also be involved in the consolidation of information in memory. When subjects learn information in a drug free state, and are treated afterwards with arecoline, there is a facilitation of information later recalled.

Amphetamine treatment also increases the amount of information which can be recalled following various modes of input processing under drug state conditions. Unlike cholinergic manipulations, amphetamine appears to amplify or strengthen trace events in memory rather than increasing the total amount of learning (size of the pool of trace events in memory). In a series of studies, it has been possible to show that amphetamine produces an enhancement of some components of cognition (in depressed patients, hyperactive children, normal children, and normal adults). Amphetamine also induces a change in state which serves as a state-specific context biasing how information is interpreted and remembered. Amphetamine treatment, like cholinergic treatment, produces state-dependent retrieval. The contrasting enhancing effects of cholinergic agents and amphetamine and the cognitive disrupting effects produced by scopolamine vs. lithium have served as one strategy for exploring the specific psychobiological mechanisms that may define different components of cognitive processes.

While alcohol has been viewed as one type of pharmacological manipulation that reliably produces learning and memory impairments in man, recent work from our laboratory in collaboration with NIAAA has demonstrated that post-processing manipulations (including treatment with alcohol) can in fact produce some enhancements in learning and memory. The focus on the biological and psychological events that follow the initial acquisition of information has generally been ignored in studies of cognitive processes. This consolidation phase of memory and the biological events that occur during this time may be important in establishing permanent records of experience. This is evident both in our studies using alcohol, where we saw evidence for a paradoxical enhancement of what has been stored in memory, and in the effects of vasopressin on reversing retrograde amnesia following ECT administration. We noted that alcohol, when administered after the processing of information, produces an enhancement in recall when tested in the unintoxicated state. This has been interpreted as an effect on memory consolidation. Other related findings suggest that alcohol induces a brief excitatory phase (possibly mediated by changes in catecholamine activity) which affects memory consolidation. This excitatory phase may be important in defining some of the reinforcement properties of alcohol. This paradoxical cognitive facilitating effect of alcohol, administered during a consolidation phase of memory, appears to highlight the differentiated mechanisms and components that make up information processing, memory, learning, and retrieval.

We have now completed a series of cholinergic trials in Alzheimer's patients and have demonstrated that cholinergic antagonists such as scopolamine mimic many of the characteristics that are evident in progressive idiopathic dementia. We have also noted that combinations of cholinergic agonists do in fact produce small but reliable enhancements of some aspects of learning and memory in patients with Alzheimer's disease. The limiting factor here has been that the extent to which an enhancement in learning and memory is evident is largely a function of the degree to which cognitive functions are preserved in these patients.

Some of our most recent studies have also demonstrated the specific role of the dopamine system in modulating effort demanding cognitive processes but not information processing that can be accomplished automatically. We have demonstrated that when older normal volunteers are treated with L-dopa (in combination with carbidopa) memory for effortfully processed events is enhanced while memory for automatically processed information is unaltered. This finding has important implications for our basic scientific understanding of the psychobiological processes that determine cognition. At the same time these findings can be translated into appropriate clinical applications in the treatment of cognitive improvements.

In another set of studies we have also begun to explore the role of the serotonin system in learning and memory. We have shown that the drug Zimelidine (a relatively specific 5 HT reuptake blocker) can substantially reverse the commonly seen cognitive impairing effects of ethanol. This finding is also important because of its basic scientific implications as well as its clinical value in the treatment of alcohol related cognitive impairments.

Summary

The recently completed programmatic-research efforts of the Unit on Cognitive Studies has extended our knowledge of the psychobiological structure and determinants of cognition. Completed research has been valuable in better defining disordered mood, the nature of the information processing impairments in progressive dementia, Korsakoff's disease, and other alcohol-related disorders, and the nature of cognitive defects in learning disabled children. Neuropharmacological studies in both unimpaired subjects and patient groups have provided us with further information about the neurochemical events important for learning, memory, and cognition. These studies have also provided new approaches in the treatment of various forms of cognitive disturbances.

Specifically, we have begun to describe the major psychobiological differences and relationships between recent (episodic) memory and knowledge (semantic memory). This distinction between these two types of memory systems is important for our understanding of forms of cognitive failure in man. We have developed a way of characterizing the nature of the cognitive changes in depression and Parkinson's Disease, and used this as a way of examining the psychobiological distinction between automatic and effortful episodic memory-learning processes. It has been possible to model forms of cognitive impairments in man such as those

that are seen in Alzheimer's disease, in drug studies of unimpaired subjects (cholinergic antagonists). This type of research has helped us in our efforts to facilitate aspects of cognition in these patients using cholinergic agonists. Neuropeptides have also been used to treat some of these cognitive disorders (e.g., arginine vasopressin). We have also modeled disorders of information processing in unimpaired subjects (with the use of naloxone). Based on our recent studies that involve changes in the dopamine and serotonin system we are also able, for the first time, to differentially affect automatic vs effortful cognitive operations and to alter recall of weak memory traces in contrast to well learned information in memory. As a result of these research efforts it seems important that we focus our new efforts in the following areas:

(a) mediational processes that are involved in transforming and encoding information (using psychophysiological and neuropharmacological tools); (b) the relationship between the reward system and memory processes, particularly as they would alter memory consolidation; and (c) new ways of facilitating impaired cognitive processes.

Significance to Biomedical Research and to the Program of the Institute

These research efforts have a direct bearing on how diagnoses of cognitive dysfunction are accomplished and the directions of future efforts for treating the cognitive impairment associated with a wide variety of psychiatric and neuropsychiatric disorders.

o

Proposed Course

We hope that current studies will lead to better diagnostic tools and effective therapies for cognitive dysfunction.

Publications

Kaye, W., Sitaram, N., Weingartner, H., Ebert, M., Gillin, J.C., and Smallberg, S.: Modest facilitation of memory in dementia. Biol. Psychiatry 17(2): 275-279, 1982.

Weingartner, H., Cohen, R., Bunney, Jr., W.E., Ebert, M.H., and Kaye, W.: Memory-learning impairments in progressive dementia and depression. Am. J. Psychiatry 139(1): 135-136, 1982.

Weingartner, H., Langer, D., Grice, J., and Rapoport, J.: Acquisition and retrieval of information in amphetamine treated hyperactive children. Psychiatry Research 6: 21-29, 1982.

Lowenstein, R., Weingartner, H., Gillin, J.C., Kaye, W., and Ebert, M.H.: Disturbances of sleep and cognitive functioning. Neurobiology of Aging 3: 371-377, 1982.

Kaye, W., Weingartner, H., Gold, P., Ebert, M.H., Gillin, J.C., Sitaram, N., and Smallberg, S.: Cognitive Effects of Cholinergic and Vasopressin-Like Agents in Patients With Primary Degenerative Dementia. In Corkin, S (Ed.): Alzheimer's Disease: A Report of Progress. New York, Raven Press, 1982, pp. 443-453.

Weingartner, H., Kaye, W., Smallberg, S., Cohen, R., Ebert, M.H., Gillin, J.C., and Gold, P.: Determinants of Memory Failures in Dementia. In Corkin S. (Ed.): Alzheimer's Disease: A Report of Progress. New York, Raven Press, 1982, pp. 171-176.

Weingartner, H., and Silberman, E.: Models of cognitive impairment. Psychopharmacol. Bull. 18(2): 27-42, 1982.

Weingartner, H.: Psychobiology and cognition. Biol. Psychiatry 3: 283-284, 1982.

Nee, L.E., Polinsky, R.J., Eldridge, R., Weingartner, H., Smallberg, S., and Ebert, M.H.: A family with histologically confirmed Alzheimer's disease. Arch. Neurol. 40: 203-208, 1983.

Strupp, B., Weingartner, H., Goodwin, F.K., and Gold, P.W.: Neurohypophyseal Hormones and Cognition. In D. De Wied (Ed.): International Journal of Pharmacology and Therapeutics and In D. De Wied (Ed.): International Encyclopedia of Pharmacology and Therapeutics (in press).

Weingartner, H., Rudorfer, M.V., Buchsbaum, M.S., and Linnoila, M.: Effects of serotonin on memory impairments produced by ethanol. Science (in press).

Weingartner, H., and Parker, E.S.: Memory Consolidation: A Cognitive Perspective. In Weingartner, H., and Parker, E.S. (Eds.): Memory Consolidation. Hillsdale, New Jersey, LEA Press (in press).

Parker, E.S., and Weingartner, H.: Retrograde Facilitation of Human Memory by Drugs: In Weingartner, H., and Parker, E.S. (Eds.): Memory Consolidation: Towards a Psychobiology of Cognition. Hillsdale, New Jersey, LEA Press (in press).

Weingartner, H., Grafman, J., Boutelle, W., Kaye, W., and Martin, P.: Forms of memory failure. Science (in press).

Weingartner, H., and Parker, E.S.: Memory Consolidation. Hillsdale, New Jersey, LEA Press (in press).

Duara, R., Rapoport, S., Weingartner, H., et al.: Resting cerebral glucose utilization, as measured with Positron Emission Tomography, in 21 healthy men between the ages of 21 and 83 years. Brain (in press).

Weingartner, H., and Silberman, E.: Cognitive Impairments in Depression. In Post, R., and Ballenger, J. (Eds.): Neurobiology of Mood Disorders. Baltimore, MD, Williams and Wilkins (in press).

Weingartner, H.: Towards an analysis of cognitive failures. Arch. Gen. Psychiatry (in press).

Weingartner, H., Gold, P., Hubran, C., Smallberg, S., and Strupp, B.: Effect of Neuropeptides on Cognition in Unimpaired Subjects: Theoretical and Methodological Implications. In Riseberg, B. (Ed.): Neuropeptide and Hormone Modulation of Brain Function and Homeostasis. Raven Press, New York (in press).

Weingartner, H.: Altered Cognitive Processes In Children: Psychobiological Approaches. In Shopsin B., and Greenhill, L. (Eds.): The Psychobiology of Childhood: Profile of Current Issues. New York, Spectrum Publications, Inc. (in press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00500-04 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cognitive and Perceptual Changes in Affective Illness

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Edward K. Silberman, M.D., Guest Worker, LPP/NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Clinical Neuropharmacology Branch, Laboratory of
Clinical Science, NIMH; Hypertension-Endocrine Branch, NHLBI; Epilepsy Branch,
NINCDS

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.0

PROFESSIONAL:

2.0

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☐ (b) Human tissues☐ (c) Neither☐ (a1) Minors☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The purpose of this project is to investigate the cognitive and perceptual changes which are present in, and characteristic of, major affective illness. The present investigation comprises five separate ongoing studies: (1) psychomotor and psychosensory symptoms in affective illness, patients with complex partial seizures, and patient controls; (2) perception and recall of emotional and neutral stimuli in depression; (3) hypothesis testing in depression; (4) lateralized hemispheric function in depression; and (5) relationship of cognitive dysfunction to diagnostic subtype and neuroendocrine abnormalities in depression.

Project Description

The premise of this project is that there are, in addition to the well-known mood changes, important alterations in perceptual and cognitive processes in major affective illness. Such symptomatology may have important implications for the pathophysiology of affective disease and may provide significant contributions to delineating clinically and prognostically homogeneous subtypes of this condition. The present study is composed of eight investigations, looking at various aspects of the problem. They are aimed at documenting the nature and extent of cognitive and perceptual changes at the clinical level, and of investigating the degree and structure of such deficits in the laboratory. Below is a summary of the current studies within this project.

1. Psychomotor and Psychosensory Symptoms in Affective Illness

A wide range of behavioral and perceptual manifestations, many with localizing significance in the brain, have been described as concomitants of complex partial seizures. The literature and clinical observation suggest that there may be areas of overlap between these phenomena and symptoms of major affective illness. The purpose of the present study is to investigate the nature and degree of such overlap. In collaboration with Dr. Robert Post (BPB), a structured interview has been devised to elicit presence of symptoms related to perception, thought processes, orientation, memory, and involuntary motor behavior. The interview is based on a survey of the literature describing such changes in epilepsy. Design of the project involves administering the interview to 120 patients; 40 are patients with a history of major affective illness currently being treated by units of the Biological Psychiatry Branch; 40 are patients with a firm diagnosis of complex partial seizures, under the care of the Epilepsy Branch, NINCDS (in collaboration with Dr. Roger Porter); and 40 are patients of the Hypertension-Endocrine Branch, NHLBI, screened for absence of psychiatric or neurological disease. Collection of data is now complete and analysis is under way. At the present time, the major hypothesis of the study appears to be confirmed. Affectively ill and epileptic patients were both found to have elevated incidence of transient visual, auditory, and olfactory changes, including both illusions and hallucinations. Epileptic, but not affective patients had elevated frequency of gustatory, visceral, and tactile sensory phenomena, and of involuntary motor symptomatology. Affectively ill but not epileptic subjects were distinguished by presence of cognitive illusions and distortions involving time.

Proposed Course

During the past year, the initial phase of the project has been completed. The relationship of symptoms to life course of illness, personality variables, medication response, and EEG findings in affective patients has been investigated. Numbers of symptoms were not found to be related to measures of length or severity of illness, or rate of cycling, but were inversely related to age. Numbers of symptoms reported correlated positively with response to lithium and tricyclic antidepressants, but were unrelated to phenothiazine response.

Although nonspecific, diffuse slowing was noted on EEG for some of the affective patients, there was no relationship between symptoms and EEG report. At the present time, plans are being formulated to investigate these symptoms in other psychiatric conditions, and to study their relationship to commonly observed clinical features, and biological markers of affective illness. A report of findings to date has been presented at the New Research section of the 1983 American Psychiatric Association meeting. A manuscript describing the work to date has been submitted for publication.

2. Perception and Recall of Emotional and Neutral Stimuli in Depression

It is a common clinical observation that affectively ill patients often seem highly insensitive to their own internal emotional state. It is also well-known that depressed subjects perform more poorly than controls in a variety of memory tasks. The purpose of this study is to look systematically at how depressed subjects evaluate the emotional qualities of verbal material, how such evaluations change with changes in clinical status, and how they interact with the subject's ability to recall the material. Fifteen depressed subjects were given a list of 40 words to rate for degree of emotionality on a zero-to-seven scale. Half the words were high emotion words, and half low emotion words, as determined by previous studies in normals. Subjects were asked to freely remember the words after they had been rated and later to pick out the words from a list in which they are intermixed with distractors. Data collection and analysis are complete at this point. A total of 31 depressed subjects and matched controls have been tested. The major findings of the study are as follows: Depressed and normal subjects do not differ in the way they rate emotionality of words. Similarly, both emotionality and concreteness of stimuli are robust memory aids for both groups. However, while depressed subjects are less benefited by both emotionality and concreteness in their free recall of words, they are more dependent upon both stimulus qualities for memory under recognition conditions. This pattern of results suggests that despite apparently similar evaluation of stimuli, the depressed process semantic aspects of material more shallowly than normals. This result is in accord with evidence in the literature that depressed are impaired primarily in tasks demanding deep or effortful processing of material.

Proposed Course

Studies are now being planned to investigate in greater detail the nature of the processing deficit in depression. In particular, depressed patients will be asked to deeply encode stimuli by making up sentences with several target words in each. Ability to form sentences (the encoding task) will be compared to ability to remember target words, thus testing whether encoding tasks fail in depression, or whether they are relatively decoupled from memory processes. Preliminary results in normals suggest that free recall (but not recognition) is robustly aided by progressively more elaborate processing tasks.

3. Hypothesis Testing in Depression

Most cognitive research in depression has focused on memory-related impairments. The present investigation uses a learning paradigm which places relatively light memory demands on the subject, focusing instead on hypothesis formulation and testing. The task, devised by Levine, is a variation on the Wisconsin Card Sort. Subjects are presented with the Levine task, which is then repeated with our own modifications. The task involves a deck of cards with two stimuli on each card. Each stimulus has four attributes (such as color and size). The subject's task is to guess which attribute has been arbitrarily designated as "correct." Subjects are asked to point to the stimulus that they think has the chosen attribute and are given yes or no feedback on selected cards. The procedure is devised so that each administration can be scored for number of hypotheses formed, number of correct hypotheses, ability of the subject to narrow down his choices as more feedback is given, and degree of hypothesis changing or keeping after positive or negative feedback. In addition, our modification of the procedure allows discrimination between poor performance due to memory deficits, or to subjects' inability to formulate an appropriate strategy. The collection and analysis of data are now complete. Depressed subjects were found to perform more poorly on the task than controls. Two components of performance distinguished depressed and control subjects, and also accounted for significant proportions of the variance in depressive performance. These were poor "focusing," or inability to efficiently narrow down the list of possible solutions to the problem, and perseveration on hypotheses which have been disconfirmed. Such a pattern of performance bears similarities to patients with both Korsakoff's dementia and right temporal lobe lesions. While the analysis suggested that, at an elementary level, logic, memory, and attention were intact in the depressed patients, the apparent inability to coordinate these functions in a complex mental task played an important role in the depressive deficit.

4. Lateralized Hemispheric Function in Depression

This study involves lateralized tachistoscopic presentation of visual material to depressed and control subjects. The task involves the subject making "same" and "different" judgments on material that can be processed either linguistically (left hemisphere) or on the basis of form (right hemisphere). Data have been collected and analyzed on ten female depressed patients, nine normal female controls, and nine normal male controls. Normal subjects showed the expected right visual field (left hemisphere) advantage in reaction time on the obligatory linguistic task, and little lateralization on the portion of the task which could be processed either verbally or spatially. By contrast, depressed subjects showed overall left visual field (right hemisphere) advantage, which was

mostly attributable to the verbal portion of the task. Thus, the results appear to represent a shift in the location of functions as they are usually performed in normal subjects, rather than merely a change in level of activation or efficiency of the hemispheres. Such a result is congruent with a variety of studies in the literature suggesting a shift in hemispheric activity away from the left and toward the right hemisphere in depression.

Proposed Course

A battery of cognitive and laterality tests is now being planned for administration to affectively ill patients. The battery is to include memory testing, a modified version of the logical processing task described above, and lateralized tests of verbal and non-verbal cognitive processing. The subject group, to include both male and female subjects, will comprise both currently ill and recovered affective patients. A select group of patients will be followed longitudinally to state vs trait changes in lateralization and level of cognitive function. The study is planned to look for cognitive trait-markers of affective illness, and to look for cognitive abilities which might possibly be prophylactic against acute affective illness.

5. Relationship of Cognitive Deficit to Diagnostic Subtype and Neuroendocrine Changes in Depression

The purposes of this study are to attempt to examine cognitive dysfunction in depression as a function of diagnostic subtype. The study is being run in collaboration with the Evaluation Unit at the Psychiatric Institute, Washington, D.C., headed by Dr. Steven Targum. Classification procedures for depressed subjects include complete DSM III diagnosis, as well as biological indices provided by the dexamethasone suppression test and the TSH response to thyrotropin releasing hormone (TRH). Cognitive testing was designed to examine not only level of performance but the structure of deficits. A battery of six memory tests were used for this study. The tests assessed the effect of type of processing (deep vs. shallow), type of recall (free vs. cued), type of stimuli (high vs. low emotional, high vs. low imageable), and level of organization of the stimuli. Level and structure of cognitive performance were examined as a function of diagnosis and also in relation to underlying metabolic abnormalities. A total of 27 depressed patients and 16 matched controls have been tested. As a whole, the depressed patients tended to perform below the level of normal controls. Depressed subjects were dichotomized according to normal vs. abnormal response to dexamethasone and thyrotropin releasing hormone (TRH) and according to presence or absence of DSM III diagnosis of Melancholia. The three methods of dichotomization proved independent in this sample. While cognitive performance did not differ according to presence or absence of Melancholia, or response to TRH, dexamethasone response did have implications for cognitive function. Those who failed to normally suppress cortisol following dexamethasone (escapers) were indistinguishable from normals in their memory performance, while dexamethasone suppressors showed the typical depressive deficit. Dexamethasone escapers were also distinguished from suppressors on a

number of structural parameters relating to memory. Escapers and suppressors did not differ in age or level of education, or on measures of depression, anxiety, hopelessness, or atypicality. That a biologically abnormal subgroup of depressives may be intact cognitively is an unexpected and challenging finding. Two explanations are that (1) hypothalamic-pituitary hyperactivity in depression has an enhancing effect on memory; or (2) the metabolic deficit in dexamethasone escapers is a mechanism of depression which bypasses cognitive functioning, while other types of depression may be more cognitively related. Some evidence for the latter hypothesis was provided by the observation that ratings on the Beck Hopelessness Scale correlated significantly with memory performance in the suppressor but not the escaper group.

Proposed Course

A manuscript is now being prepared for publication. Plans are being formulated to attempt to replicate the findings in other depressed populations.

Significance to Biomedical Research and to the Program of the Institute

These investigations are a part of the program of basic research at NIMH arrived at elucidating the nature of affective illness. Cognitively related studies are relevant to this goal from three points of view: (1) they concern an important area of deficit in affective illness, (2) they define an aspect of dysfunction which may provide clues to the pathologic anatomy and physiology of affective illness, and (3) they may provide useful information relating to clinically meaningful classification of affective disorders.

Publications

Silberman, E.K., Weingartner, H., Laraia, M., Byrnes, S., and Post, R.M.: Processing of emotional properties of stimuli by depressed and normal subjects, J. Nerv. Ment. Dis. 171: 10-14, 1983.

Silberman, E.K., Weingartner, H., and Post, R.M.: Thinking disorder in depression: Logic and strategy in an abstract reasoning task. Arch. Gen. Psychiatry, 40: 775-780, 1983..

Silberman, E.K., Weingartner, H., Stillman, R., Chen, Hong-Jen, and Post, R.M.: Altered lateralization of cognitive processes in female depressed patients. Am. J. Psychiatry, in press.

Silberman, E.K., Post, R.M., Nurnberger, J., Theodore, W.: Epileptic-like symptoms in affective illness. New Research Abstracts, No. NR68, 136 Annual meeting of Am. Psychiatric Assoc., 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00502-04 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Atypicality in Major Depressive Illness		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Edward K. Silberman, M.D., Guest Worker, LPP, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.0	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input checked="" type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> To explore the possibility of an important <u>atypicality</u> dimension within the group of <u>primary, endogenous depression</u>, we have constructed a <u>rating scale</u> for <u>atypical depressive illness</u>. <u>Forty-four NIMH patients</u> were rated, all meeting Research Diagnostic Criteria for primary, major depressive illness. <u>Atypicality</u> in this group was characterized by <u>lack of encapsulated episodes, interpersonal difficulties, evidence of narcissistic character disorder, and high anxiety, and somatization</u>. Though atypical patients were <u>younger</u> than typical, they were <u>hospitalized significantly more often</u>. Biologically, they had <u>smaller variance</u> in two measures of <u>norepinephrine metabolism</u>, as well as <u>lower levels of platelet MAO</u>. <u>Typicals were significantly more likely to have an antidepressant response to sleep deprivation than atypicals</u>. Thus, atypicality as a dimension within primary, major depression may have important theoretical and clinical implications. </p> <p style="text-align: center;"> This project was terminated 10/1/82. </p>		

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00503-03 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Human Clinical Studies of Attention Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Allan F. Mirsky, Chief, LPP, NIMH		
COOPERATING UNITS (if any) Epilepsy Branch, Clinical Neurosciences Branch, NINCDS; Laboratory of Clinical Science, NIMH; University of Virginia, Boston University		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.75	OTHER: 1.25
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> This research comprises three related areas of investigation concerned with specifying <u>neuropsychological</u> factors underlying clinical conditions in humans in which <u>disturbed attention</u> is a major symptom. A major emphasis is on (1) illuminating the nature of brain stem pathophysiology, if any, in such entities as <u>petit mal</u> or <u>absence epilepsy</u>, <u>infantile autism</u>, <u>schizophrenia</u>, and related diseases; (2) an additional major emphasis is on extending the neuro-behavioral analysis of attention loss in absence epilepsy so as to facilitate developing alternative treatment strategies for such patients. Both of these projects form part of a larger effort which is aimed at (3) developing a comprehensive and systematic <u>taxonomy</u> of <u>attentional disorders</u> in humans. This latter study will eventually comprise study of patients with <u>cerebral lesions</u>, <u>seizures</u>, <u>dementing diseases</u>, and <u>metabolic illnesses</u> of the brain. </p>		
(621)		

Other Professional Personnel

Connie C. Duncan-Johnson, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH
 Richard Coppola, D.Sc., Senior Engineer Officer, LPP, NIMH
 Herbert Weingartner, Ph.D., Chief, Unit on Cognitive Studies, LPP, NIMH
 Theodore P. Zahn, Ph.D., Research Psychologist, LPP, NIMH
 Richard Nakamura, Ph.D., Senior Staff Fellow, LPP, NIMH
 Roger Porter, M.D., Chief, EBB, NINCDS
 Judy Rumsey, Ph.D., Staff Fellow, LCS, NIMH
 Fritz Dreifuss, M.D., Professor of Neurology, University of Virginia
 Debbi Fein, Ph.D., Asst., Professor of Psychiatry (Neuropsychology), Boston Univ.

Project Description1. Brain Stem Mechanisms in Attention Impairment

Current approaches to the neuropsychology of attention impairment have emphasized that the system responsible for the maintenance of attention or consciousness within the brain is most likely represented at a variety of levels of the neuraxis. From an evolutionary point of view, it is clear that the capacity for sustained attentive behavior is present in many species which do not possess more than a rudimentary forebrain or telencephalon. MacLean's analysis of the R-complex within the human brain leads to the view that this "clump of ganglia," which constitutes virtually all of the reptilian brain, can support a variety of ritualistic, repetitive behaviors which could be characterized as sustained and attentive. Evolution progressed and the brain developed additional complexity and volume. Additional capacity for attentive behavior was thus overlaid on the more primitive, although in many aspects thoroughly adequate, brain stem system of the reptile. Therefore, although the system for maintenance of attentive behavior in the human (or higher primate) includes limbic and neocortical components, the brain stem remains a key component and possibly the keystone of the entire system. Authors such as Hughlings Jackson and Penfield and Jasper recognized this in their conceptions, respectively, of "highest level seizures" and the "centrencephalon." In their theorizing, consciousness was either localized in or regulated by deep brain stem structures. Without reviewing all of the evidence that led to those views of the hierarchical organization of attention and consciousness within the brain, we nevertheless point to the extremely deleterious effects on such capacities of small lesions in the brain stem region of the third and fourth ventricles. In the last ten years, a new technological refinement of evoked potential methodology has made possible an other-than-theoretical exploration of the role of brain stem structures in certain clinical states. This "far field" technique makes it possible to assess the integrity of auditory (and somatosensory) relay nuclei within the brain stem of humans. Although the technique has probably had most utilization in the diagnosis of demyelinating disease, it has also been used in the study of other neurological and, recently, psychiatric disorders. There may or may not be any specific interest in these sensory systems (auditory, somatosensory) in studying a particular clinical entity (i.e., absence seizures, infantile autism); nevertheless, the possibility of evaluating the functional integrity of certain systems within the brain stem is extraordinarily valuable, and many clinical investigators are using these techniques. We have published work indicating that

there are disturbances (prolonged transmission time) in the processing of auditory information in the brain stem in infantile autism. We have also shown that in absence seizures (spike-wave activity), both naturally-occurring and experimentally-induced, there may be perturbations of auditory brain stem functioning. We are planning to continue such studies with these patient groups and others once our facilities have been developed in the ACRF.

2. Neurobehavioral Studies in Absence Epilepsy

We have for a number of years been studying the absence attack in patients with petit mal/centrencephalic/absence seizures (the terms are more or less interchangeable) as a model state to understand the phenomenon of consciousness/attention. Some of these studies have involved comparing the behavioral capacities of patients suffering from petit mal--as opposed to focal seizure disorders; other studies have involved detailed comparison and contrast between the behavioral and the electroencephalographic symptoms/signs of the disorder. Most recently these investigations have: (1) used evoked potentials in the visual and auditory modalities as indices of the sensory effects of generalized seizure activity of the symmetrical and synchronous spike and wave (SW) variety, and (2) examined changes in the EEG power spectrum prior to SW bursts as prodromal signs which may be used to predict (and ultimately to control) SW bursts. We propose to continue this line of neurobehavioral investigation, using event related potentials of various types as well as other behavioral and physiological tools, to refine further our understanding of the nature of altered consciousness in absence (petit mal) epilepsy.

3. A Taxonomy of Attentional Disorders

The goal of this project is to develop a comprehensive and coherent account of the relation between symptoms of altered or disturbed attention or consciousness as they appear in various clinical entities, the other behavioral and clinical characteristics of the several disorders, and the specific central nervous system damage or disturbance in each disorder. The attentive capacities of the patients will be assessed by a number of measures comprised within the GAT (generalized attention test) which is an outgrowth of the CPT (continuous performance test) a measure of sustained visual attentive behavior. The ultimate goal will describe the precise attentive deficit (as opposed to cognitive losses) and the nature of the neuropathophysiology associated with each of the following clinical entities:

- cerebral cortical lesions (frontal, parietal, or temporal lobe)
- centrencephalic/absence epilepsy
- schizophrenia
- infantile autism
- dementing diseases (Alzheimer's, Korsakoff's, Huntington's).

We will attempt, as well, to relate these changes where possible to standardized measures of mnemonic and other cognitive function, and to autonomic indices of attention, arousal, and habituation.

The technique of mapping EEG and evoked potentials developed by Coppola and Buchsbaum has been refined to such an extent that it can now form an integral part of these attentional studies. It is hoped that these maps will provide an additional measure to relate to behavioral and other indices of pathophysiology.

Significance to Biomedical Research and to the Program of the Institute

Since attention disturbance is a characteristic of many significant psycho- and neuropathological disorders, it is essential to have a clear empirical and theoretical account of the role and pathophysiological significance of the symptom. It will aid in understanding the etiology and course of these illnesses and may aid in improving their treatment.

Proposed Course

We have run a small group of schizophrenic, epileptic, and brain-injured patients through our laboratory procedures (i.e., CPT, brain stem auditory evoked potentials, various tests of cognition and memory, autonomic indices of attention, etc.). During the course of the next year, we hope to recruit additional cases from other diagnostic categories into this taxonomic study. However, since we have not had a laboratory to pursue this work, it has not been possible to achieve substantial progress during this reporting period.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00504-03 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Models in the Monkey of Generalized Seizures of the Absence Type		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Allan F. Mirsky, Chief, LPP, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">0.6</div>	PROFESSIONAL: <div style="text-align: center;">0.6</div>	OTHER: <div style="text-align: center;">0.0</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Generalized seizure activity with the electrographic appearance of <u>absence epilepsy</u> (bilaterally symmetrical and synchronous paroxysmal three-per-second spike and wave discharges) can be elicited in the <u>monkey</u> by a variety of methods. These include electrical <u>stimulation</u> of various locations within the brain, injection of <u>convulsant drugs</u> and other substances, and administration of compounds which may alter normal inhibitory mechanisms within the cell. Model seizure states created in these ways are studied in order to test hypotheses about pathophysiological seizure mechanisms, sensory processing and attentional capacities during absence seizures, effects of spike-wave activity on cellular activity, and effects of techniques or maneuvers which may modify or reduce convulsive activity. Most recently this project has involved the following work: we studied the (paradoxical) seizure inducing effects of a GABA-enhancer and the effects on auditory brain stem evoked potentials of generalized seizures induced by injection of pentylenetetrazol.</p>		

Other Professional Personnel

Eva Bakay Pragay, Ph.D., Research Psychologist, LPP, NIMH

Project Description

γ -vinyl GABA and γ -acetylenic GABA are two recently synthesized compounds whose metabolic effects include the blocking of the enzyme action responsible for the metabolism of the inhibitory neuro-transmitter GABA. The accumulation of GABA thus produced should have an anticonvulsant action, and so it does, at moderate doses of these compounds. However, as the dose is increased, there is a paradoxical rebound effect and animals treated with large quantities of either γ -vinyl or γ -acetylenic GABA have shown paroxysmal seizure activity. And of interest to us is the fact that the seizure activity is not the clinically obvious generalized tonic-clonic variety. Instead, although widespread spikes and spike-wave patterns may be seen, there may be few clinical signs. Such an effect is reminiscent of absence seizures (staring spells) in human centrencephalic epilepsy. We are in the process of exploring the utility of these compounds for producing model seizures of the petit mal variety in the monkey.

We have also induced generalized seizure activity in rhesus monkeys, reflected in both clinical and EEG manifestations, by systemic administration of pentylenetetrazol. Brain stem auditory evoked potentials (BAEP) were recorded from indwelling epidural electrodes at the vertex of the skull as well as from electrodes implanted along the primary auditory pathway in the brain stem (inferior colliculus). Several components of the complex "far field" vertex potential showed increased latency and decreased amplitude during ictal episodes as compared to control periods both pre-drug and post-seizure. Similar changes were seen in direct recordings from brain stem auditory structures. The parallel recording of "far field" (vertex) potentials and "near field" (brain stem auditory pathway) potentials appears to be a fruitful approach. The direct recording from brain stem auditory structures adds reliability and temporal resolution to the findings. Thus, in contrast to the vertex potential which requires several hundreds, or even thousands of stimulus repetitions, only a few samples are necessary to obtain reliable waveforms from direct brain stem recordings. Consequently, the grain and the resolution of the experiment can be enhanced, and various periods of pre-, during- and post-ictal stages as well as phases of gradual recovery can be assessed. The analysis of small consecutive samples revealed profound BAEP changes not only during the ictal period but immediately following the seizure activity. There was also marked fluctuation of suppression and potentiation during the post-ictal recovery period.

Significance to Biomedical Research and to the Program of the Institute

This experiment provides direct evidence of brain stem involvement in consciousness and in generalized seizures and contributes to the current efforts to produce an accurate primate-based model of the pathophysiological processes in absence epilepsy.

Proposed Course

We will be continuing with this experimental program as primate facilities become available to LPP. We can report no progress on this project during the past year since we had no laboratory available to pursue this work.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00505-03 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Lesion and State Change Effects on Visual Attention		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Richard K. Nakamura, Ph.D., Senior Staff Fellow, LPP/NIMH		
COOPERATING UNITS (if any) Laboratory of Neuropsychology, Laboratory of Cerebral Metabolism, and Adult Psychiatry Branch, NIMH		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 10px 0;"> This project consists of four related areas of investigation, all concerned with the analysis of mechanisms of <u>attention</u>. Special emphasis is placed on the use of <u>lesions</u>, <u>physiological recording</u>, and <u>metabolic mapping</u> techniques to elucidate mechanisms involved in visual attention. The four areas are: (1) attention and cerebral mechanisms of visual behavior; (2) pharmacological mechanisms of attention; (3) brain activity in inattention; glucose metabolism and protein synthesis in sleep; and (4) physiological studies of selective visual attention. </p>		

1. Attention Mechanisms and Visual Behavior

We have found that large nonvisual lesions of cerebral cortex in the monkey cause permanent blindness. The basic preparation is as follows: one hemisphere is visually deafferented by combined optic tract transection and forebrain commissurotomy. The other hemisphere has all cortical areas removed except for the striate, prestriate and inferior temporal visual areas. Animals prepared in this way were functionally blind for over a year despite anatomical evidence of an intact visual system. We have been analyzing this phenomenon in an effort to determine the role of nonvisual cortex in visual behavior and to establish the nature of higher processing in the visual system. The major results indicate that we are beginning to elucidate the role of attention in sensory processing.

We have examined the single neuron responses to visual stimuli in the visual systems of our blind monkeys. Results of over 400 neurons studied to date in the blind animals compared to over 200 neurons in normal animals indicate that visual responses of the blind are near normal in both striate and prestriate areas. Preliminary data from inferior temporal cortex, on the other hand, suggest that the blind animals show less specificity of neuronal response than seen in the normals. These data imply that in the absence of feedback from higher cortical structures and in the absence of behavioral feedback, the visual system continues near normal processing through to the prestriate cortex. Only at the level of inferior temporal cortex does the effect of such feedback losses change cellular responses to visual stimulation. This in turn suggests that the effects of attention on neuronal responses will not be significant in areas earlier in the visual pathway than inferior temporal cortex.

In an effort to determine the critical cortical zones of this blindness phenomenon, the large nonvisual ablation has been subdivided into three parts: the sensorimotor cortex, the limbic cortex, and the polysensory cortex. These areas have been ablated in separate groups of monkeys and the only lesion which produces a blindness effect is the polysensory cortical lesion. This area consists of dorsal prefrontal cortex, inferior parietal cortex, and superior temporal cortex (including insula). It is of considerable significance to us that a neglect or inattention syndrome follows the ablation of any portion of the polysensory area for this suggests that our animals cannot see because they cannot attend to the visual modality.

The absence of effect of the sensorimotor lesion creates confidence that the blindness is not simply the result of a disconnection of visual input from motor output. In the original chronic blindness preparation, the non-visual lesion is placed in one hemisphere, the optic tract to the other is cut and the forebrain commissures are divided. If, however, the forebrain commissures are left intact, then there will be a period of blindness lasting approximately 10 to 40 days. This period is followed by a recovery of visual function which permits not only visual guidance to food, but discrimination of visual patterns as well.

This recovery of visual function, when a path of communication is left between the hemispheres indicates that the blindness is the result of a disconnection of vision from higher cortical processing areas. Combined with

earlier results, we can be more specific and say that the blindness is caused by a disconnection of visual areas from polysensory (attentional) areas of the brain. Thus a connection between visual and attentional areas appears to be critical before visual behavior can proceed in monkeys.

2. Pharmacologic Mechanisms of Attention

Striatal dopamine has been implicated as an important transmitter in the attention-arousal system. Areas A9 and A10 (substantia nigra pars compacta and the ventral tegmental area) have been shown to be major sources of dopamine for the brain in general and the cerebral cortex in particular. In rats, lesions of areas A9 and A10 have been associated with inattention or neglect, tremor, transient aphasia and adypsia, and a deficit on the delayed alternation task. Little is known about the effects of such ablations in the monkey though in man cell loss and reduced dopamine in these areas have been associated with Parkinsons disease.

Because of our interest in striatal dopamine, we have begun a study with the Division of Special Mental Health Research, Adult Psychiatry Branch, to investigate the possibility of transplanting dopamine secreting tissue into the brains of monkeys that have been previously deprived of striatal dopamine with unilateral 6-hydroxydopamine ablations. Objectives of the research are to: a) look at the effects of unilateral ablations of dopamine systems b) examine the practicability of brain tissue implants in the monkey, and c) see if behavioral changes following dopamine ablations can be reversed with transplanted tissue. Preliminary data have shown that it is possible to transplant fetal substantia nigra tissue into a monkey brain and get both growth and dopamine secretions, but because of the tremendous cost of fetal monkey tissue and the problem of immune system reactions we are now transplanting dopamine secreting adrenal medulla removed from the subject monkeys. Grafts have survived transplantation for over six months.

Unilateral substantia nigra lesions in monkeys have small effect in the period immediately following surgery and this effect soon disappears. A longer lasting deficit may be expected to follow bilateral substantia nigra lesions produced serially so now we plan to use this preparation to determine if dopamine tissue grafts can produce recovery of functions (and we are particularly interested in attention deficits) lost after the substantia nigra lesions.

3. Brain Activity in a State of Inattention

Major clues to the systems involved in attention might be derived from the study of natural states of reduced attention such as sleep. In collaboration with the Laboratory of Cerebral Metabolism, the Laboratory of Neuropsychology, and the Sleep Laboratory, we have been studying cerebral glucose metabolism of monkeys in slow wave sleep and wakefulness. A total of eight monkeys have been examined, four experimental animals in slow wave sleep and four control animals that were kept awake. The major finding has been that the animals in sleep show an overall reduction in cerebral metabolism of about 30%. Further, we have been unable to find any brain structure which shows, on average, higher activity in

slow wave sleep that in the awake state. Hypnogenic center theories, which postulate a brain area which actively keeps an animal in sleep, are therefore not supported. The loss in attention which accompanies slow wave sleep thus appears to be the result of a general loss of brain activity.

In addition, we have applied a new method to determine local cerebral protein incorporation in another 8 monkeys, four in slow wave sleep and four that were awake. Preliminary data from these animals show that protein synthesis, like cerebral glucose metabolism, is reduced throughout the brain in slow wave sleep. Theories of sleep suggesting that protein synthesis increases during sleep to make up for deficits incurred during wakefulness are therefore contradicted.

We will next examine the effect of paradoxical or REM sleep on brain activity. REM sleep is of particular interest because the organism is in a state of total inattention while at the same time the brain is electrographically very similar to the awake brain. Thus the brain structures controlling the difference in attention state may be more readily revealed.

4. Physiological Mechanisms Underlying Visual Selective Attention

While the foregoing studies may reveal important clues to the nature of attention, they all represent inherently indirect approaches because in none is a systematic attempt made to isolate the attentional process while controlling for other processes such as sensory input and motor output. If sensory input and motor output were held constant while attention was made to switch from one aspect of the sensory input to another, then it would be possible to directly study the mechanisms of attention.

We have designed a general purpose test which will allow such a direct examination of attention. Included are additional design features such as: a) the task will be usable for both humans and monkeys; b) it will accommodate both electrophysiological and behavioral analysis by permitting quantification of attention changes and allowing precise timing of brain events associated with these changes; and c) information will be transmitted through the visual system to take advantage of the great information available on visual processing.

Event related potentials will be recorded using scalp electrodes on humans and using invasive indwelling electrodes in monkeys to determine the topography of attention related potentials in the brain.

Significance to Biomedical Research and to the Program of the Institute

We hope to understand the mechanisms underlying attention and consciousness in animals and man. This will enable us to develop adequate animal models of

human clinical syndromes featuring reduction of attention or consciousness, such as schizophrenia, dementia, and petit mal epilepsy.

Proposed Course

All these studies are in progress and several papers marking stages of development are in preparation.

Publications

Nakamura, R.K., Coates, R., Crawford, H., and Friedman, D.: A flexible restraint chair for the Cynomolgus monkey (Macaca fascicularis). J.Med. Primatol. 11: 178-185, 1982.

Nakamura, R.K., Kennedy, C., Gillin, J.C., Suda, S., Ito, M., Storch, F.I., Mendelson, W., Sokoloff, L., and Mishkin, M. Hypnogenic center theory of sleep: no support from metabolic mapping in monkeys. Brain Res. 268: 372-376, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00506-03 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Attention-Related Neurons in the Brain of the Rhesus Monkey

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Eva Bakay Pragay, Ph.D., Research Psychologist LPP/NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

3.2

PROFESSIONAL:

2.2

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

This project is concerned with an analysis of the activity of nerve cells in that system within the primate brain which is necessary and responsible for the process we refer to as attention. Monkeys trained to perform visually-guided go, no-go discrimination tasks are tested whilst extra cellular recordings are made from brain regions thought to be part of an attentional system. The most recent study in this series examined structures in the forebrain.

We have found attention-related units in the anterior portion of the upper bank of the cingulate sulcus and in the periaruate area of the dorsal frontal cortex. These cells responded to the manipulation of attention, e.g., manipulation of the pre-stimulus waiting period or changing the behavioral significance of the task-stimuli. These attention-related units have properties similar to those found in the brainstem reticular formation in previous studies by Bakay Pragay, Mirsky, Ray, and colleagues.

Project Description

In an effort to describe at a cellular level the brain system in the primate necessary for attentional behavior, we have developed a monkey preparation that permits such study. Extra-cellular recordings are made from trained animals while they perform on discrimination tasks requiring visual attention. In earlier work, we described "attention-related" cells in the mesopontine region of the brainstem. Later work has been aimed at forebrain areas extending laterally from the rostral bank of the arcuate sulcus to the central sulcus and extending medially to the cingulate sulcus. More recent work was devoted to the reassessment of findings in the anterior prefrontal regions; the exploration was focussed on the pre-arcuate and peri-principalis cortex and corresponding peri-cingulate areas.

As in prior studies, the task required the animal to press a "hold" button for 2 seconds in order to turn on a "cue" button; the latter was transilluminated by either a red ("go") or a green ("no go") cue-light. In the go trials, the animal had to release the hold button and press the cue button within 1 second. In the no go trials, it had to maintain pressure on the hold button for another second. In the basic task, both correct go and no go trials were rewarded.

The task permitted us to distinguish response-related (Type I) and stimulus-related (Type II) cell types. Type I units, which responded only during go trials, can be regarded as related to the execution of the instrumental motor response. Type II neurons which respond during both go and no go trials could be related to various functions, which could be defined by their temporal relationship to various task-events (events in the trial), as well as by applying variations in the experimental conditions. Type II activity could be classified into the following subgroups: (1) post-stimulus, pre-reinforcement change; (2) anticipatory activity starting in the intertrial interval, usually in the form of a gradual increase in firing rate of the cell preceding stimulus onset ("pure" anticipation), or in addition, following stimulus onset (combined anticipatory and "evoked" activity); (3) pre-reinforcement activity. Post-stimulus (evoked) activity, whether in pure or combined form, could be symmetrical in shape and in magnitude for both go and no go trials, or asymmetrical, the latter occurring mostly in the form of the go-trial-related response being bigger (more intensive and/or more systematic and/or longer lasting) as compared to the no go trial-related response.

The attention-related property of the Type II units was tested by varying the within-task conditions. These included: (a) reward for both correct go and no go trials (basic task); (b) non-reinforcement for correct no go trials (NRNG); (c) non-reinforcement for correct go trials (NRG); and (d) varying the length of the fixed intertrial interval (ITI): 1 sec, 2 sec (basic task), and 3 sec (or 4 sec). In addition, two extra-task conditions were applied: visual stimuli (including the task-stimuli) without access to task or reward, and non-contingent delivery of reward. The NRNG condition and the extra-task administration of stimuli represented manipulation of attention through variation of the reward value of the stimuli. Variation of the length of the ITI manipulated attention by varying the possibility of development of a preparatory set.

The results indicate a difference in the distribution of various types of cells along the anterior-posterior dimensions of the forebrain areas described above. Units in the more anterior regions (in the periarculate area) plus in the corresponding levels around the cingulate sulcus showed the following characteristics: Type II was predominant over Type I; the Type II activity was predominantly anticipatory and/or symmetrical for both go and no go trials. Type II units responded readily to the manipulation of attention: Increasing of the pre-event waiting period (e.g., to the onset of stimulus, to the offset of stimulus or to the gratuitous reward delivered in regular intervals) induced or increased anticipatory activity to these events. Decreasing the significance of the task-stimuli by displaying them outside of the task-and-reward context decreased or eliminated peristimulus cell-activity. Pre-saged omission of reward in the task-context decreased cellular activity following stimulus onset; unexpected omission of reward induced phasic increase of activation in some cells for both go and no go trials.

On the other hand, more caudal regions around the central sulcus and the corresponding cingulate area showed predominantly Type I, post-response activity. These units did not respond to the variation of task-conditions described above.

In the intermediate areas (dorsal convexity at the level of the superior precentral sulcus) Type II and Type I cells were equally numerous. Type II units in this area were typically "asymmetrical," showing more intensive and/or longer lasting response in go trials. The no go response, although small, did not deteriorate during homogenous no go series, unlike the "erratic" no go response of some extremely asymmetric units seen in more caudal regions.

The anticipatory activity, seen in some asymmetrical units was smaller (less intensive and of shorter duration) as compared to the symmetrical units. In some non-anticipatory asymmetrical units, homogenous blocks of go trials induced a slight anticipatory-preparatory activity.

Functional Significance

Concerning the functional significance of the various forms of Type II activity, the following propositions could be made: The symmetrical anticipatory activity in the anterior forebrain may represent a sensory preparatory set facilitating performance in an attention task. In addition (or alternatively) these units may convey information about discrepancy between expected and actual time course of external events. The asymmetrical Type II units may be considered visuo-kinetic in the sense that they get activated in preparation to visually guided and or timed behavior. They may form a link between attentional processes of the anterior forebrain and motor commanding functions of the precentral motor cortex.

Significance to Biomedical Research and to the Program of the Institute

This study is an important step forward in our program of attention research. It represents a second step in the line of exploring various brain regions, by

means of extra-cellular unit recording techniques in a go no-go task. Any final assignment of function must, of course, await histological confirmation of the electrode locations. However, this work provides an important basic research underpinning for our clinical studies of attention disorders.

Proposed Course

We hope to complete this portion of the study of the attentional function of the prefrontal cortex over the next year.

Publications

Mirsky, A.F. and Bakay Pragay, E.: Brain mechanisms in the processing of sensory information: Clinical symptoms, animal models, and unit analysis. In D.E. Sheer (Ed.): Attention: Theory, brain functions, and clinical application. Hillsdale, N.J., Erlbaum, in press.

Ray, C.L., Mirsky, A.F., and Bakay Pragay, E.: Functional analysis of attention-related unit activity in the reticular formation of the monkey. Exp. Neurol. 77: 544-562, 1982.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00507-01 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Clinical Brain Imaging

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Robert M. Cohen, M.D., Ph.D., Acting Chief, CBI, LPP, NIMH

COOPERATING UNITS (if any)

Department of Nuclear Medicine, CC, NIH; Neuroscience Branch, NIMH; Biological Psychiatry Branch, NIMH; Dept. of Psychiatry, University of California at Irvine

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

Section on Clinical Brain Imaging

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

6.5

PROFESSIONAL:

6.0

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Investigators in this section have begun to refine existing methodologies for the study of cortical functioning on the basis of positron emission tomography (PET) and electrical brain mapping procedures in humans.

As wide variability has been noted in normals and patient groups in terms of PET determinations of cerebral glucography, additional effort has been forthcoming to control for behavioral variability and psychological task in these studies. This has included the use of somatosensory stimulus paradigms.

Using some previously available PET methodologies and some new approaches, schizophrenic and affectively disordered patients appear to differ from normals. Psychiatric patients appear to have somewhat lower ratios of frontal to posterior cortical rates of glucose metabolism. However, the interpretation of these findings is obscure as these ratios do not represent an absolute lowering of glucose metabolic rates in the frontal cortex of psychiatric patients. Other preliminary findings suggest that schizophrenic patients appear to have lower glucose metabolism rates in the left central gray matter structures of the brain but elevated metabolism in both temporal lobes.

By electrophysiology, a diminution of the N120 component of the somatosensory evoked potential has been observed in normals in response to a series of similar somatosensory stimuli. This habituation which is most prominent in somatosensory area II does not appear to occur in schizophrenic patients.

Other Professional Personnel

Allan F. Mirsky, Ph.D., Chief, LPP, NIMH
 Lynn DeLisi, M.D., Staff Psychiatrist, LPP, NIMH
 Henry H. Holcomb, M.D., Clinical Associate, LPP, NIMH
 Richard Coppola, LPP, NIMH
 Monte Buchsbaum, M.D., Department of Psychiatry, U. of California at Irving
 David Pickar, M.D., NSB, NIMH
 John Boronow, M.D., NSB, NIMH
 Robert M. Post, M.D., Chief, BPB, NIMH
 Thomas W. Uhde, M.D., BPB, NIMH
 Robert M. Kessler, M.D., CC, NIH
 Richard Margolin, M.D., CC, NM, NIH
 John I. Nurnberger, M.D., BPB, NIMH
 John Morihisa, M.D., APB, NIMH at St. Elizabeths Hosp.
 William Carpenter, M.D., Spring Grove State Hosp., U. of Md., Dept. of Psych.

Objective

The goals of this project are to develop and apply methods for imaging the brain based on its functional characteristics so as to further our understanding of normal and abnormal human behavior.

Methods Employed

Behavioral Assessment

A detailed assessment and screening of all normal volunteers and patients participating in projects in the section occurs. This includes a structured interview, the Cannon-Spoor social adjustment scale, a detailed alcohol and drug history, family history and medical and psychiatric histories. Where appropriate, the Hamilton and Beck Depression scales, BPRS, the Strauss/Carpenter outcome scale, the global assessment scale, the AIMS (for motor movement ratings), and Krawiecka scale, to rate positive and negative symptom clusters in schizophrenia, are used. All subjects rate themselves using the Spielberger Anxiety Scale to report their experience during actual imaging procedures.

Biological Assessment

In conjunction with other laboratories subjects are often assessed for dexamethasone suppressibility, TRH responsiveness, serotonin platelet uptake, and drug treatment responsiveness. In addition, blood, urine and cerebrospinal fluid measurements reflecting neurochemical activity are in the future expected to be utilized in conjunction with electrophysiologic and PET data.

X-ray transmission tomography (CT Scan) is used for measurements of ventricular size, sulcal atrophy and hemispheric asymmetry. Positron Emission Tomography (PET) is also used. PET, utilizing similar reconstruction mathematics as that of CT scanning, enables the user to obtain slice images of radioisotope cortical location. Using F18-2-deoxyglucose (FDG) as the radioisotope tracer and the methods developed by Sokoloff and others, it is possible to obtain data

on local glucose metabolism and consequently, probable local cortical functional activity.

Mapping of Electrical Activity

If a large number of electrodes are used, the recordings of EEG and evoked potential can also be used to develop images or maps of cortical activity, albeit of surface topology. Presently, 12 standard 10/20 system points on the left hemisphere and midline, and four additional points between existing posterior leads are used. This method offers the potential of tracking behavioral events in the millisecond range in comparison to the 30' of integrated cortical activity which the PET Scan displays.

Major Findings

Method Development

PET Scans result in a rate of glucose utilization. Methods for appropriate, accurate, and noninvestigator biased analysis of the enormous data accumulated by this method are required. Initial analyses were performed as mean counts per picture element as a method was not available for converting FDG-isotope counts to glucose metabolism rates. The methodology is currently available for this calculation.

As our chief interest in behavior-brain-relationships lie in gray-matter structures we wanted to develop methods of analysis which were based on trying to isolate these structures from the rest of the skull and its contents. The first attempt in this direction was to analyze the gray matter mantle of each cortical slice. To do this, Dr. Buchsbaum and John Cappelletti used a thresholding method similar to those developed for the CT Scan to outline the brain contour. The center of the brain was then located by fitting a straight line, using a least squares approach, to the midpoints of a series of horizontal lines that connected the left and right sides of the slice outline. The outer view of the brain slice, assured to be mainly the gray matter of the cortex, was then determined by connecting the brain outline and corresponding center point by a radial scan procedure thereby producing a 2.3 cm size strip within and bordering the brain outline. Most of the analyses reported in other sections were accomplished by using this computer derived cortical peel as divided into two segments for each quadrant of the slice. Current problems with this analysis include difficulties accounting for head rotation, lack of correction for variation in cerebral gray mantle thickness or for the partial voluming effects resulting from the limited resolution of the scanner, that is unwanted contributions from skull and white matter adjacent to the gray matter contributing to the isotope count obtained by this procedure. New methods are currently under investigation for these problems which include utilizing a program based on a differential method of locating the peak of the Gaussian distribution of isotope counts expected from a rim of cortical gray matter. A method has also been developed by Drs. Buchsbaum, Cappelletti and Coppola for making lateralized maps of this gray mantle distribution for direct comparison to EEG data.

Patient Studies with PET

Presently, 22 schizophrenic patients, 15 affective disordered patients and 43 controls have been studied.

In initial studies of 8 schizophrenic patients and 6 normals, subjects sat resting in an acoustically-treated darkened room with eyes closed following injection of 3.5 mCi FDG. Patients with schizophrenia showed lower ratios of mean glucose metabolism rates of specific quadrants of the frontal cortex to mean values of the slice, and secondly, a relatively low uptake of FDG in the left central gray-matter region, consisting primarily of the caudate nucleus.

As this work might have represented a differential in the activation of the frontal lobes in normals as compared to schizophrenic patients, in a second series of patients, somatosensory stimuli were administered to the right forearm. This method, as utilized in prior pain experiments in the lab, had demonstrated observable differences between normals and schizophrenics. Also, Dr. Ingvar and associates had previously noted increased frontal blood flow in another pain paradigm, the placement of subjects' hands in a bucket of ice water. Sixteen patients with schizophrenia, 11 patients with affective disorder and 19 normals have now been analyzed. All groups demonstrated an anteroposterior gradient in glucose metabolism especially at superior slice levels. Both patient groups showed less of an anteroposterior gradient which represented primarily higher metabolism rates in posterior regions, rather than lower levels in frontal regions. A 4-way ANOVA (groups by slice level by hemisphere by section) revealed no significant group or groups interaction effect. Front-back ratios for right side were significant for groups only with 1-tailed t-tests. Normal subjects (1.08, SD. = 0.11) vs. schizophrenics (1.02, SD. = 0.08) was ($t = 1.78$, $p < 0.05$, 1 tailed) and for left side, the differences of 1.11 for normals vs. 1.06 for schizophrenics did not reach statistical significance. No significant differences were observed between affective disorder and schizophrenia groups.

Using an edge finding computerized program, schizophrenic patients were found to have elevated glucose use in both temporal lobes compared to controls; hallucinators were found to have greater ratios of left to right temporal lobe metabolism compared to non-hallucinators or controls. In addition to directing this latter effort, Dr. DeLisi has begun examining CAT scans to look for correlations with PET findings in collaboration with Dr. Margolin, Dr. Boronow and Mr. DeLeo. So far, findings of hypofrontality in schizophrenia have not correlated with cerebral atrophy on CT Scans.

Human Studies with Electrical Mapping

Using a 16 lead evoked potential mapping system, Dr. Henry Holcomb has been directing the study of somatosensory responses to pain stimuli in normals and schizophrenic patients. Ongoing work from our laboratory has demonstrated that affectively disordered and schizophrenic patients are relatively pain insensitive by the pain discrimination measurement initiated by Dr. Buchsbaum in the laboratory. A decreased N120 component of the somatosensory evoked potential is found in schizophrenic patients. In dividing the 35 normals into those with high

vs. low CSF opiate receptor binding levels, those with low β -endorphin equivalent levels were more sensitive (11.9 vs. 11.1) than those with high β -endorphin equivalents. A similar phenomenon was observed for 24 depressed subjects (13.8 vs. 7.3).

Following mildly painful electrical stimuli of 16 mA for 1 msec duration, in normals there appears to be a gradual diminution in the N120 (the negative potential component of the somatosensory evoked potential occurring between 112-148 msec post-stimulation). There is a negative correlation ($r = -0.44$, $p < 0.05$) between this diminution and pain sensitivity as determined by a pain discrimination task. In comparison to the 16 normals tested, 15 unmedicated schizophrenic patients showed a constant value or sometimes a rise in this component. The level of habituation correlated significantly with BPRS ratings ($r = -0.57$). These differences were most marked in what we believe to be somatosensory area II of the cortex.

Significance to Biomedical Research and the Program of the Institute

In the past, there have been considerable difficulties involved in trying to assess region specific functional activities as well as neurotransmitter functional activity in the brain. PET Scanning and mapping based on electrical activities provide two very exciting approaches for solving these methodologic problems. They promise to play particularly important roles in the study of psychiatric disorders where there is little evidence for structural changes in the brain. The findings so far have already begun to elucidate some aspects of the normal physiology of pain pathways and the possible psychiatrically related alterations in the same.

A concentrated effort has also been made to examine frontal cortex activation in normal and psychiatric patients as measured by the PET Scan as the function of this anterior area of the cortex may relate more closely to observed alterations of behavior in psychiatric patients. In the past, the increased blood flow observed in this region upon task initiation has been hypothesized to result from the presumed responsibility of this region for the planning of goal-directed behavior in people in comparison to posterior cortical areas which may relate more directly to sensory processes. The capacity then to assess specific illness and task related deficits in activation provide us with an exciting and challenging tool for the elucidation of the mechanisms that might underlie abnormal behavior.

Proposed Course

We need to continue to improve our methodology. PET Scan analyses utilizing FDG need to take advantage of the improved calculations that a 4 constant model affords. This is a model which takes into account the loss of isotope counts that results from the liberation of trapped FDG by phosphatase in the brain. In addition we need to implement more automated noninvestigator biased methods of data analysis.

There is the need to develop tests that assure that populations to be compared are actually being compared without artifact. For example, a continuous

performance task in which you are more likely to be able to control the subject's cognition and have a means of assessing this control is likely to provide a good paradigm for intergroup comparisons and to establish adequate correlations between behavior and physiology.

We also need means to assess how dependent the glucography method is to the state of the patient at the time of scan.

In evaluating the importance of PET data, most importantly, new tracers need to be developed to extend our functional mapping capacities.

We need to continue to evaluate PET findings in relation to evoked potential data, biochemical measurements, and behavioral assessments including drug response. Insofar as only small differences have been observed between psychologically disturbed patients and normals, new strategies emphasizing selective neurotransmitter challenge approaches are a logical step in the search for neurotransmitter or regional functional differences between patients and normals.

In addition, the data on somatosensory habituation should be extended to study other patient groups; e.g., phobic patients and Alzheimer patients. The electrophysiological mapping procedures facilitate the study of the psychopharmacology of habituation, a very important fundamental process of the nervous system upon which most higher cortical functions may depend.

Publications

Buchsbaum, M.S., Coppola, R., and Cappelletti, J.: Positron emission tomography EEG and evoked potential topography: New approaches to local function in pharmaco-electroencephalography. In Herrmann, W.M. (Ed.): Electroencephalography in Drug Research, West Germany, Gustav Fischer Verlag, 193-207, 1982.

Buchsbaum, M.S., Cappelletti, J., Coppola, R., Rigal, F., King, A.C., and van Kammen, D.P.: New methods to determine the CNS effects of antigeriatric compounds: EEG topography and glucose use, Drug Development Research 2: 489-496, 1982.

Buchsbaum, M.S., King, A.C., Cappelletti, J., Coppola, R., and van Kammen, D.P.: Visual evoked potential topography in patients with schizophrenia and normal controls. Adv. Biol. Psychiatry 9: 50-56, 1982.

Davis, G.C., Buchsbaum, M.S., Naber, D., Pickar, D., Post, R., van Kammen, D., and Bunney, W.E., Jr.: Altered pain perception and CSF endorphins in psychiatric illness. N.Y. Acad. Sci., 398: 366-376, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00508-01 LPP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neuropsychological Evaluation of Psychiatric and Neurological Patients

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Connie C. Duncan-Johnson, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH

COOPERATING UNITS (if any)

Biological Psychiatry Branch, Clinical Neuroscience Branch, Clinical Neuropharmacology Branch, Laboratory of Clinical Science, Adult Psychiatry Branch, NIMH; and Epilepsy Branch, NINCDS

LAB/BRANCH

Laboratory of Psychology and Psychopathology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.5

PROFESSIONAL:

0.5

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A comprehensive neuropsychological test battery has been devised to provide a complete assessment of various cognitive functions which can be related to damage or dysfunction in different regions of the brain. The battery comprises tests designed to tap the following aspects of behavior: executive functions, language, vigilance (attention), visual-spatial capacity, memory, motor behavior and auditory functioning. In addition, measures of psychometric intelligence, personality, color vision and hand and eye dominance are included. The intent of developing the battery is to provide an archival assessment of the neurobehavioral capacities of the various subgroups of patients who are studied by investigators within the LPP; although, eventually, administration of the battery may be extended to all IRP patients. The data can thus provide a complete behavioral assessment against which to relate the neurophysiological, neuroradiological and biochemical information that is gathered concurrently on these patients. The data will be included in the permanent file of each patient and should provide eventual actuarial summarization of cognitive and perceptual functions for the different clinical populations that have been studied in the IRP and facilitate research relating to behavioral factors in neuro- and psychopathology.

Other Professional Personnel

Allan F. Mirsky, Ph.D., Chief, Laboratory of Psychology and Psychopathology, NIMH
 Elkhonon Goldberg, Ph.D., Associate Professor of Psychiatry, Albert Einstein
 College of Medicine
 Robert Post, M.D., Chief, Biological Psychiatry Branch, NIMH
 David Pickar, M.D., Chief, Section on Clinical Studies, Clinical Neuroscience
 Branch, NIMH
 Dennis Murphy, M.D., Chief, Clinical Neuropharmacology Branch, NIMH
 Michael Ebert, M.D., Chief, Section on Experimental Therapeutics, Laboratory of
 Clinical Science, NIMH
 Richard J. Wyatt, M.D., Chief, Adult Psychiatry Branch, NIMH
 Roger Porter, M.D., Chief, Epilepsy Branch, NINCDS

Objectives

There are several goals of this project: (1) To provide a standard, comprehensive neurobehavioral assessment of all IRP patients for archival and actuarial purposes. As such, the data gathered will form part of the permanent record for each patient and will facilitate current and future research relating to behavioral factors in neuro- and psychopathology. (2) To provide a complete behavioral description of various patients who are also being studied in conjunction with various research protocols but, more specifically, the protocols aimed at developing a taxonomy of attention disorders. The behavioral data can then be correlated with the neurophysiological, neuroradiological and biochemical data which are concurrently being gathered on these patients. (3) To provide neurobehaviorally-defined subgroups that might reduce variability in psychiatric diagnosis, treatment and outcome.

Methods Employed

The neuropsychological battery includes the tests listed below. Intellectual and sensory functions are presented in tabular form along with the test(s) used to assess them.

FUNCTION MEASUREDTESTExecutive

Sequencing, Attention	Trail Making Test
Attention	Stroop Colour-Word Test
Perception and Reasoning	Raven's Progressive Matrices (B-E)
Concept Formation and Abstraction	Wisconsin Card Sorting Task

Language

Initiation	Verbal Fluency Test
Lexical	Boston Aphasia Test--Word Discrimination and Visual Confrontation Naming

Written

Phonemic
Comprehension

Boston Aphasia Test--

Word Discrimination

Spreen-Benton Sound Lending Test

Token Test

Goldberg's Semantic Test

Oral Apraxia

Boston Aphasia Test--Oral Agility

Vigilance, Attention

Continuous Performance Test

Visual-Spatial

Raven's Progressive Matrices (A)

Hooper Visual Organization Test

Witkin's Embedded Figures Test

Butter's Embedded Figures Test

Memory

Global

Recent Verbal Memory

Remote Verbal Memory

Recent Visual-Spatial Memory

Wechsler Memory Scale

Buschke Selective Reminding Test

Boston Remote Memory Test

Kimura's Recurrent Figures Test

Motor Functions

Purdue Pegboard

Boston Apraxia Test

General IntelligenceWechsler Adult Intelligence Scale--
RevisedPersonalityMinnesota Multiphasic Personality
InventorySensory and Perceptual

Visual Acuity

Color Vision

Hand Dominance

Eye Dominance

Kimura's Dichotic Medodies

Major Findings

Ms. Kathleen Squillace has been trained to administer the test battery, and pilot testing is complete. To date, we have collected data on 12 patients, including cases with major affective disorders, eating disorders and seizure disorders. There are insufficient data to draw any inferences or conclusions at this time.

Significance to Biomedical Research and the Program of the Institute

The data base we are providing will constitute a permanent, archival behavioral assessment of the neuropsychological capacities of all patients seen

by investigators in the LPP and, eventually, (it is hoped) of all IRP patients. It should provide an invaluable resource for all current and future studies in the LPP (and IRP) in which the goal is to relate behavior and physiology, whether pathological or normal. Of particular value and interest will be the correlations to be drawn in our taxonomy of attention research.

Proposed Course

We will continue to test all patients studied by investigators in the LPP to the extent that resources and time permit. As the sample size increases and data accumulate, we will be able to construct neuropsychological test profiles for the different clinical populations under study and to begin to interrelate the behavioral, neurophysiological, biochemical and neuroradiological domains of information. If the success of the program warrants it, and if resources are available, the administration of the battery may be extended to include all IRP patients.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00509-01 LPP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Attention Disorders As Assessed by Event-Related Brain Potentials		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Connie C. Duncan-Johnson, Ph.D., Chief, Unit on Psychophysiology, LPP, NIMH		
COOPERATING UNITS (if any). Biological Psychiatry Branch, Clinical Neuroscience Branch, Clinical Neuropharmacology Branch, Laboratory of Clinical Science, Adult Psychiatry Branch, NIMH; and Epilepsy Branch, NINCDS		
LAB/BRANCH Laboratory of Psychology and Psychopathology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.6	PROFESSIONAL: 0.6	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input checked="" type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> The major purpose of this project is to investigate the roles of <u>event-related brain potentials</u>, <u>attention</u> and <u>information processing</u> and their <u>interrelationships</u> in the etiology, pathology and prognosis of psychiatric and neurologic disorders. Major emphasis is on the diagnostic specificity of disorders of attention and cognition and identification of the specific stage(s) of information processing underlying observed decrements in performance. Concurrently recorded event-related brain potentials and performance on cognitive tasks are used to define mechanisms of attention failure in subjects with diagnoses of <u>schizophrenia</u>, <u>affective disorders</u>, <u>seizures</u>, <u>dementing diseases</u>, <u>attention deficit disorder</u>, <u>learning disorders</u>, <u>eating disorders</u>, <u>infantile autism</u> and <u>cerebral lesions</u>. Biological processes influencing event-related brain potential activity are investigated by testing the effects of drugs and other treatments and by correlating these variables with biochemical measurements and those derived from radiological cerebral mapping. Psychological correlates are investigated by relating the data to extensive neuropsychological, psychiatric and personality measures. </p>		

Other Professional Personnel

Allan F. Mirsky, Ph.D., Chief, Laboratory of Psychology and Psychopathology, NIMH
Robert Post, M.D., Chief, Biological Psychiatry Branch, NIMH
David Pickar, M.D., Chief, Section on Clinical Studies, Clinical Neuroscience Branch, NIMH
Dennis Murphy, M.D., Chief, Clinical Neuropharmacology Branch, NIMH
Michael Ebert, M.D., Chief, Section on Experimental Therapeutics, Laboratory of Clinical Science, NIMH
Richard J. Wyatt, M.D., Chief, Adult Psychiatry Branch, NIMH
Roger Porter, M.D., Chief, Epilepsy Branch, NINCDS

Project Description

A. Objectives

The major objective of this project is to yield data that will contribute to a taxonomy of attention disorders and to relate this to the clinical disorders of schizophrenia, epilepsy and other forms of brain pathology. Defining the specific ways in which information processing can fail may provide new diagnostic strategies for more effective evaluation and treatment of patients with attentional and cognitive impairments. We propose to use concurrently obtained event-related brain potentials, or "ERPs," and measures of performance during active cognitive processing to begin to define the mechanisms of attention failure in these syndromes. Defining and understanding the different determinants and forms of attentional and cognitive failure is diagnostically important, as well as useful in characterizing the nature of the psychobiology of attention disorders.

B. Methods Employed

1. Electrophysiological Assessment

The general methods of these studies include recording the EEG, utilizing the International 10-20 system of electrode placement, while subjects perform a variety of tasks. Tasks include tests of auditory and visual attention and memory and use reaction time techniques and/or recall and recognition of stimulus material. The EEG is averaged to yield ERPs that provide information on the attentional and cognitive functioning of the subject. A mini-computer system is used to run the experiments and to collect and analyze the data.

2. Neuropsychological Assessment

A detailed assessment will be conducted on all patients and normal volunteers. This includes a standard structured interview which yields detailed data on alcohol and drug history, family history and medical and psychiatric histories. Moreover, each subject will be evaluated on an extensive neuropsychological battery of intellectual functioning. Where appropriate,

verbal and nonverbal tests of formal thought disorder and the Krawiecka scale of symptoms in schizophrenia will be administered.

3. Biological Assessment

In collaboration with other laboratories, subjects will be assessed for drug treatment responsiveness. Blood, urine and cerebrospinal fluid measurements reflecting neurochemical activity will be used in conjunction with electrophysiological and neuropsychological data.

X-ray transmission tomography (CT scan) will be used to measure ventricular size and positron emission tomography (PET) will be used to obtain data on local glucose metabolism. These data will be correlated with electrophysiological data to yield information on the relation between ERPs and the structure and functional activity of the cortex.

Significance to Biomedical Research and the Program of the Institute

Since attention and cognitive deficit are characteristic of many prominent psychopathological and neuropathological disorders, it is important to develop a precise empirical and theoretical account of these symptoms. The scalp-recorded ERP is the only noninvasive approach available to study rapidly changing neural activity associated with cognitive processing in human subjects. The ERP provides information on mental events involved in selective attention, stimulus evaluation, memory, learning and response preparation. For example, the latency of one ERP component, the "P300," has been shown to index the timing of cognitive operations involved in the evaluation of a stimulus, independently of the multiplicity of factors that determine the total duration of an overt response. This method thus offers the potential of tracking behavioral events in the millisecond range. The appropriateness of evaluating ERPs in studies of attention is apparent, as they may provide a dissection of the various components involved and thereby permit more precise identification of the type of information processing deficit responsible for poor performance on attention tasks in a variety of patient groups. Moreover, because ERPs can provide information independently of overt responses, they are especially useful for studying patients in whom overt behavior may be altered or impaired.

Proposed Course

Collection of data will commence for projects on schizophrenia, epilepsy and other psychopathological and neuropathological disorders, with the goal of determining the relation of ERP variables to diagnosis, diagnostic symptomatology, severity of psychosis, degree of formal thought disorder, performance on tests of attention and memory and intellectual functioning, degree of improvement during treatment and improvement on specific treatments. ERP measures in patient groups will be studied in relation to data obtained from CT and PET scans as well as biochemical assays of body fluids such as monoamines and their metabolites in cerebrospinal fluid. Electrophysiological predictors of clinical response to neuroleptic medications will be sought.

Since we have not had a laboratory to pursue this project, it has not been possible to achieve substantial progress during this reporting period. We have, however, begun pilot testing and plan to launch this program of research once software development is complete.

Publications

Duncan-Johnson, C.C., Roth, W.T., and Kopell, B.S.: Effects of stimulus sequence on P300 and reaction time in schizophrenics: A preliminary report. In Karrer, R., Cohen, J., and Tueting, P. (Eds.): Brain and Information: Event-related Potentials. New York, The New York Academy of Sciences, in press.

Ford, J.M., Duncan-Johnson, C.C., Pfefferbaum, A., and Kopell, B.S.: Expectancy for events in old age: Stimulus sequence effects on P300 and RT. J. Gerontol. 37: 696-704, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00672-18 LSES
PERIOD COVERED October 1, 1982, to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Social Psychological Correlates of Occupational Position		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. L. Kohn, Chief, Laboratory of Socio-environmental Studies, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Socio-environmental Studies		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 12	PROFESSIONAL: 5.25	OTHER: 6.75
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The object of this study is to assess the <u>reciprocal effects</u> of occupational <u>conditions</u> and <u>psychological functioning</u> (in particular, values, self-conceptions, social orientation, and intellectual flexibility). Structured interviews were conducted in 1964 with a sample of 3101 men, representative of all men employed in civilian occupations throughout the United States. The study was extended into a <u>longitudinal study</u> in 1974, with the reinterviewing of a randomly-selected one-fourth of the original sample, together with their wives and, where appropriate, one of their children. <u>Replications</u> of this research have been carried out in <u>Poland</u> and <u>Japan</u> .		

Other:	C. Schooler	Research Psychologist	LSES	NIMH
	J. Miller	Guest Worker	LSES	NIMH
	K. Miller	Research Sociologist	LSES	NIMH
	K. Slomczynski	Visiting Scientist	LSES	NIMH
	W. FitzGerald	Research Sociologist	LSES	NIMH
	K. Tominaga	Visiting Scientist	LSES	NIMH
	C. Schoenbach	Social Science Analyst	LSES	NIMH

Project Description:

The principal goal of this research is to assess the relationships between people's job conditions and their psychological functioning. The evidence thus far provided by this research demonstrates that job conditions have a marked impact on cognitive functioning, on values, and on conceptions of self and orientations to society.

The research began in 1964 with structured interviews with a sample of 3100 men, representative of all men employed in civilian occupations throughout the United States. These interviews were conducted to Melvin Kohn and Carmi Schooler's specifications by the National Opinion Research Center (NORC) of the University of Chicago. In 1974, NORC conducted follow-up interviews, again to Kohn and Schooler's specifications, with a randomly selected one-fourth of the men who had participated in the original survey. Wherever a man was found to be presently married, a nearly identical interview was separately conducted with his wife. And wherever a man had one or more children in the age-range 13 through 25, a similar interview was conducted with a previously selected child.

One major purpose of the follow-up study has been to provide more definitive data about causal processes than could be provided by a single cross-sectional survey. With these data, the investigators have attempted to assess the magnitudes of the reciprocal effects of job conditions and several important facets of psychological functioning. The study of wives was designed to ascertain whether job conditions affect men and women similarly. The research has shown that they do. These data may also enable the investigators to assess the effects of men's job experiences on their wives' psychological functioning and of women's job experiences on their husbands' psychological functioning, in each case taking account of the individual's own job experiences. The study of the children was designed for exploratory analyses of the effects of parental experiences, values, and practices on their children's psychological development, as well as of the children's own educational and occupational experiences on their own psychological development.

By the end of FY1982, the investigators had completed the manuscript of a volume reporting two decades of research on the interrelationship of social stratification, job conditions, and adult psychological functioning. [This is discussed in some detail in the Annual Report for FY1982.] This year required considerable further investment of time in the actual production of the book -- proof-reading, editing, and the preparation of a comprehensive, analytic index -- but the main intellectual work on that phase of research had been completed. There still remains considerable

further work on cross-national validation of the American findings, particularly for Japan, and on testing whether the overall findings apply to theoretically pertinent subgroups of the population. (For example, is the relationship between job conditions and psychological functioning similar for people at differing stages of career and life course?) Still, the main thrust of the research has now shifted from the effects of social structure on adult psychological functioning to the effects of social structure -- including, but not limited to, those effects that are transmitted through the family -- on the psychological development of children.

During this past year, the major research efforts have been addressed to the following activities: a general reconceptualization of the processes by which values are transmitted from one generation to the next; a rather large-scale process of "tooling up" for analyses to come (mainly in the development of new indices); two principal lines of concrete data-analysis -- one on the relationship between educational experience and children's personality development, the other on the transmission of values in the family; and continuing cross-national comparative analyses, for Poland and Japan, of the relationships between job conditions and adult psychological functioning. This Annual Report will summarize all of these activities. It will also briefly summarize a more general interpretation of the psychological consequences of environmental complexity, developed by Carmi Schooler out of the analyses he has carried out in the course of this research.

RECONCEPTUALIZING THE INTERGENERATIONAL TRANSMISSION OF VALUES

Our strategy has been to redefine a classic sociological problem -- the transmission of values in the family -- by treating value-transmission in its larger social-structural context. We see the transmission of values in the family as part of a much more general process by which social structure affects children's values and orientations, with the family as only one -- albeit a crucial -- institutional mechanism in this process.

By values we mean conceptions of the desirable -- criteria of preference. In this usage, we are following Robin Williams's treatment of values. By parental values, we mean the values that parents would most like to see embodied in their children's behavior -- the characteristics they consider most desirable to inculcate in their children. Parent-to-child value transmission implies, at minimum, that children come to hold the same fundamental values for themselves that their parents think desirable for them. Most research on the transmission of values in the family has focused on the magnitude of the correlation between parents' and children's values rather than on the process of value-transmission within the family or on the part that the family plays in the larger process of socializing the child in society. Most investigators seem to have begun with the assumption that there would be a high correlation between parents' and children's values and have been surprised to find the correlations to be much lower than they had expected. Strong parent-child correlations

have been found for such things as political orientations, religious beliefs, and "life styles", but until now, not for values as such or even for general orientations to social reality. Studies of values have consistently found rather modest levels of agreement -- correlations of roughly 0.15 to 0.25 -- between parents and children. But past studies have generally dealt with valuation of quite specific characteristics -- for example, honesty or obedience -- rather than with fundamental dimensions of values -- such as valuation of self-direction versus conformity to external authority. Moreover, these studies have not taken unreliability of measurement into account. Our analyses, to be discussed below, do take measurement error into account and do find substantially higher parent-child correlations.

The much more important issue, though, is that even a very high correlation between parents' and children's values would not necessarily mean that parents' values have been transmitted to their children. We have every reason to believe that the socialization process is more complex than that. Parent-to-child value transmission is embedded in a larger social context, a context that is to some extent deliberately designed by parents but is to some extent beyond parental control, often beyond parental knowledge. Moreover, while it is true that parents and children experience largely similar social environments, it is also true that their environments diverge in significant ways. Not only do parents and children spend much of their time doing different things in different institutions, but, as Morris Rosenberg showed, the same social-structural conditions may impinge on adults and children in decidedly different ways.

Such complex processes are not going to be understood just by asking, "What is the zero-order correlation between parents' and children's values?" or even, "What is the partial correlation between parents' and children's values, statistically controlling social-stratification position, sex of parent, sex of child, and whatever else seems relevant?" On the contrary, it will be necessary to develop causal models in which pertinent variables will not be "statistically controlled" in the partial-correlational sense, but in which their direct and indirect influences will be assessed as part of an ongoing process. We should ask how parent-to-child value transmission fits into a much more general process whereby a child's conditions of life, both those that are intentionally and those that are inadvertently imposed by parents, affect that child's values.

This perspective can be made more explicit by a preliminary and highly schematic structural model of the process of value transmission (see the Figure). Although schematic, and certainly incomplete, the model can serve as a basis for designing research and guiding data analysis on the process of value transmission as it operates in a single society -- the United States or any other industrialized society. Presumably, the parameters of any such model would differ in different societies and the model would have to be enlarged for comparative analysis. Such issues as the influence of parental values on children's values in societies that actively seek to

weaken that influence through competing social agencies, for example, the schools or the mass media, as compared to societies that make no such efforts, can be addressed, in time, through comparative research.

Our model of value transmission is basically analogous to Blau and Duncan's reformulation of the issue of social mobility in their book, The American Occupational Structure. Mobility had previously been measured by one or another index that expressed the degree of similarity of parental status and offspring's achieved status; this we see as analogous to the correlation between parents' and children's values in research on the transmission of values in the family. Blau and Duncan decomposed that single measure of similarity or difference, using parents' status as one of the independent variables in a causal model of offspring's attained status. Rather than simply assessing the amount of stability or mobility, they explicated the process of mobility. Finally, they (and to an even greater extent, subsequent investigators who developed the model further, e.g., Sewell, Hauser, and Featherman) showed how the process of intergenerational mobility is embedded in a larger social process of status attainment. Following their example, we use parental values as one of the determinants of children's values in a more general causal model of the process by which children "attain" their values. But, for several reasons, a model of value transmission must be more complex than the Blau-Duncan model of status attainment.

First, parental values cannot be treated as the beginning of the process, as parental status was taken as the beginning of the process in the original Blau-Duncan model, but must be seen as intervening in a chain that starts with the social-structural conditions of parents' lives, particularly (but certainly not only) their positions in the hierarchical ordering of the society. This is the part of the model on which we have concentrated most of our efforts thus far. Carmi Schooler's, Carrie Schoenbach's and Melvin Kohn's studies of longitudinal data for the United States, and Kazimierz Slomczynski's, Joanne Miller's, and Melvin Kohn's comparative analyses of data for Poland and the United States, provide firm evidence about the causal interrelationships of education, occupational position, job conditions, and parental values. In particular, we know that both education and the job conditions that facilitate or inhibit the exercise of occupational self-direction -- namely, the substantive complexity of work, routinization, and closeness of supervision -- have a decided impact on parental valuation of self-direction versus conformity to external authority. We have also found that parents' values, in turn, affect parents' job conditions -- presumably because parents' values for their children reflect their more general values, for themselves as well as for their children, and people's values do affect how they perform their jobs. The relationships between occupational self-direction and parental values are therefore shown in the Figure to be reciprocal.

The second issue is so obvious as to be embarrassing to raise, yet it has received surprisingly little attention in the research literature: Mothers and fathers often do not have the same values. Our U.S. data show,

for example, that the "true" correlation between mothers' and fathers' valuation of self-direction versus conformity to external authority, for children of both sexes aged 13-25 years, is approximately 0.52. Although substantial, a correlation of 0.52 hardly constitutes perfect agreement. When parents disagree, whose values are transmitted to the child? Does it depend on sex of parent vis-a-vis sex of child? Or does it depend on whether the child is closer to the mother or to the father? Or on other intra-family processes? Or on the social-stratification position of the family or its ethnicity? Even listing these possibilities makes it obvious that a single correlation between parents' and children's values is at best a crude average of possibly large correlations between some parent-child pairs and probably negligible correlations between other parent-child pairs. The schematic model in the Figure does not take all these possibilities into account. It does, at least, depict mothers' and fathers' values as independently derived, albeit correlated, and as independently affecting children's values.

Third, despite decades of research in developmental psychology and in the sociology of socialization, all too little is known about the processes by which parents' values are communicated to their offspring. Presumably, children infer parental values not only from parents' assertions but also from their practices. In all probability, disciplinary practices are involved, insofar as parents' values guide their disciplinary practices (as some of Kohn's earlier research showed) and these disciplinary practices exemplify for children their parents' values in action. Presumably, too, by affecting parents' more general conceptions of their responsibilities vis-a-vis their children (as some of Kohn's earlier research also showed), parental values affect the nature and quality of the parent-child relationship and, in this way too, exemplify for children their parents' values. But the very generality of these statements -- prefaced as they are by "presumably's" -- is an admission of how little is actually known. We don't even know much about the accuracy of children's perceptions of their parents' values. Nor do we know whether accurate perception of parental values is necessary for a child's coming to share the parents' values -- which is why, in the Figure, we treat children's perceptions of their parents' values as a possible but not as a necessary intervening variable from parents' to children's values.

Fourth, we shall have to conceptualize the ways in which children's outside-the-family worlds are structured. The crucial questions to ask are: "What are the immediately impinging conditions of a child's life?" and "How are these conditions socially structured?" It is particularly important to recognize that the same social-structural conditions may impinge quite differently on parents and children. For example, it has for many years been one of our principal preoccupations to elucidate the ways that the conditions of life encountered by adult men and women are affected by their social-stratification positions. Social-stratification position matters for adults in large part because of the close link between stratification position and job conditions, in particular, the opportunity to be self-directed in one's work. Occupational self-direction, in turn,

has a profound effect on values. But if Bowles and Gintis's analysis is correct, a child's opportunity to be self-directed in work -- that is, in school work -- is determined less by the family's social-stratification position than by the child's school level. In the lower school grades, children are trained to conform to adult authority; in higher school grades, the schools come more and more to train children and young adults to be self-directed. According to this view, the family's social-stratification position is pertinent not so much because children from families of different stratification levels go to different schools, but mainly because children from families of lower social-stratification position leave school earlier, when they have been trained only to conform to the dictates of authority. Children from families of higher social-stratification position stay in school longer and are trained to be self-directed.

We think that Bowles and Gintis are essentially correct in their observation and also in their insight that schools teach children to value conformity to authority or, in higher grades, to value self-direction, not by preaching at them, but by organizing their lives in ways that are conducive to their valuing one or the other. We therefore consider it necessary to think of children's work in school in much the same way that we think of adults' work in factories and offices -- in terms of its degree of substantive complexity, closeness of supervision, and the like. Karen A. Miller, Carmi Schooler, and Melvin Kohn are presently engaged in just such research. (This research is discussed in some detail below.)

For now, the Figure merely depicts "educational self-direction" as one of the "social conditions of the child's life," without showing its social-structural sources. A more complete model would have to trace such links as those between parental social-stratification position, parental values, public or private schooling, school curriculum (including the critical issue of school track), and educational self-direction.

Our treatment of other social conditions of the child's life is even more nebulous: We merely assign to a black box "the influences of siblings, peers, and neighborhood." In so doing, we acknowledge but do not really deal with the existence of that large, albeit inconclusive, literature on possible conflicts between parents' and peers' values, leaving the entire question, as well as the associated question of the social-structural sources of peers' values, out of our schema. The Figure doesn't even note the influence of television, an influence that NIMH has spent several million dollars studying. Our formulation is admittedly primitive just where we think new conceptualization is most urgently needed, in understanding the factors outside the parent-child relationship that affect a child's values.

Fifth, despite the depiction of the Figure, we cannot assume that a socialization model is necessarily additive. On the contrary, we should start with the hypothesis that the central processes are interactive, in the analysis-of-variance sense of interactive. We believe, for example,

that the closeness of parent-child relationships will prove to be important in affecting children's values, but not because a close parent-child relationship is itself conducive to the child's developing any particular value orientation. Rather, we think it likely that the closeness of parent-child relationships will interact with parental values and practices in affecting children's values, in which case, parental values would be more successfully "transmitted" to children by those parents who have close relationships with their children than by those parents who do not. Unfortunately for ease of research design, many of the components of the model may interact with other components. It may even be that parents' values will have greater or less effect on children's values depending on the type of school or neighborhood. Moreover, all of the components may affect children's values differentially, depending on the age and sex of a child -- a possibility much too complex to show in the Figure, but one that must be kept in mind throughout. Our schematic outline simply treats all relationships as if additive, it being too difficult to do otherwise in such a depiction. A systematic analysis, however, must test the possibilities of interaction throughout the model.

Sixth is a question -- or really, a set of questions -- about timing. The model is structural, not dynamic; that is, it does not deal with rates of change in the transmission of values, only with total effects. (In testing a model of this type, one would use structural equations, not differential equations or difference equations.) Even so, questions of timing enter the model in several ways. There is, to begin, the question of when to measure children's values, which may change considerably during children's formative years. Should we expect children's values to be most like those of their parents when children are quite young and presumably most subject to their parents' influence? Or later, as children develop further? Or perhaps not until they, themselves, are adults? The issue has not yet been systematically considered or addressed empirically. Moreover, children's experiences may have different impacts on their personality development depending on the stage of the life course at which the experiences are encountered. Then, too, the same experiences may matter differentially in different historical contexts (a phenomenon generally called a "cohort effect" but sometimes in this context referred to as "generational differences"). These and other time-related issues make it exceedingly unlikely that any single study of value transmission can be definitive, no matter how well it is designed. The most we can expect to achieve in any particular study is clear specification of the limits of generality of the particular study; it will take a well-designed set of studies to vary the "time" parameters systematically. In the schematic outline of the Figure, all that we have been able to include is one aspect of timing -- the question of whether it is parents' values when a child is young, as that child grows older, or some combination of the two, that affects a child's values.

Seventh, and finally, an adequate model of value transmission is made even more complex by the likelihood that children's values may not only be affected by, but may also affect, their parents' values. We have therefore

shown these effects as reciprocal in the Figure. While it is certainly possible to assess reciprocal effects empirically, such assessment is difficult and may require exceptionally rich longitudinal data. The problem is made even more complex when we recognize that values may also affect and be affected by other psychological phenomena, including intellectual functioning. This complexity we have not even dared indicate in the Figure, lest our "schematic outline of the transmission of values" become a grandiose model of the entire social psychology of the child.

In sum, the intergenerational transmission of values is a much more complex process than has generally been realized. Even calling the problem one of "transmitting" values may be so great an oversimplification as to be a misnomer. In particular, the transmission of values in the family is but one part, albeit a crucial part, of a much more general process by which a child's conditions of life in home, school, and in direct and vicarious experience of the larger social world come to shape that child's values. It is this process to which much of our current research is addressed.

TOOLING UP FOR NEW ANALYSES

A considerable amount of effort has been devoted this year to developing new indices for use in the intended analyses of social structure and children's personality development, as well as for analyses of social structure and personality more generally. Some of these efforts will be discussed below, in context of the analyses of educational experience and children's psychological development and of the assessment of the correlations between parents' and children's values. In addition, Carmi Schooler has developed indices of intra-family process (from the parents' and children's data of the follow-up study, and from fathers' data in the initial baseline study), for use in our intended analyses of the role of parent-child relationships in the intergenerational transmission of values. Kazimierz Slomczynski has similarly developed indices of intra-family process from parents' and children's data of the Polish studies. Rather than describe these indices out of context, we shall leave their description to future Annual Reports, when we can describe not only how the indices were conceptualized and developed, but also how they were used in causal analysis.

Of somewhat more general utility has been the development of a method for disaggregating the components of social mobility. In studying the relationship between social mobility and psychological functioning, one of the crucial methodological problems is determining the components of social mobility. Kazimierz Slomczynski and Tadeusz Krauze (of Hofstra University) have provided a new tool for analysing the components of social mobility. They showed that using the equation, "Circulation mobility equals total mobility minus structural mobility", in scalar rather than in matrix form, as has been done for more than two decades, limits the range of possible applications. Analyzing the generalized, matrix form of the equation, the investigators developed a satisfactory representation of traditional concepts of structural and circulation mobility fulfilling the criteria of

face validity and consistency with formal postulates. They provided a method for decomposing the matrix of observed mobility (N) into a sum of three nonnegative matrices: immobility (I), structural mobility (S), and circulation mobility (C). In the future, the decomposition, $N = I + S + C$, can be used in analyzing the effects of the components of social mobility on psychological functioning in terms of contextual variables for individuals.

EDUCATIONAL EXPERIENCE AND CHILDREN'S PSYCHOLOGICAL DEVELOPMENT

The purpose of Karen Miller, Melvin Kohn, and Carmi Schooler's analysis is to examine the processes by which students' educational experiences, particularly the degree of self-direction they are able to exercise in their educational endeavors, affect their psychological functioning. Data for this analysis were collected in the 1974 follow-up survey, when one pre-selected child of each father in the sample was interviewed. The interview schedule for these "children" -- then 13 to 25 years old -- contains an intensive battery of questions about the current educational experiences of all those respondents still in school. These questions are designed to parallel those previously found to be useful for analyzing occupational experience, focusing on such dimensions of the educational experience as its substantive complexity and how closely the student is supervised. The intent is to see whether the concepts and methods developed for the study of occupational conditions can be applied as well and with similar results to the study of educational conditions.

Previous analyses conducted by members of the Laboratory on the impact of job conditions on psychological functioning have treated work as a socializing experience; Miller, Kohn, and Schooler apply the same interpretive model to education that heretofore has been applied to occupation. The underlying hypothesis is that self-direction is important in young people's school experiences, just as in older people's job experiences. People who use initiative, thought and independent judgment in their daily work, whether in school or paid employment, will come to think more effectively not only in work but in other spheres of life. The psychological process is one of learning and generalization.

One of the presumed goals of formal education is to increase the effectiveness of students' cognitive functioning. But whether and, if so, how education achieves this goal is an issue about which there has been much debate. Part of this year's work was devoted to examining this issue by refining and further testing a causal model, developed in preliminary form last year, of the reciprocal effects of educational self-direction and cognitive functioning.

Opportunities for educational self-direction are, to a substantial degree, built into the structure of the educational system. The investigators assume (and test in their analysis) that there is a direct relationship between grade-level and the exercise of educational self-direction, with students at higher grade levels having both greater

opportunity and greater need to exercise educational self-direction. Opportunities for educational self-direction also may be affected by other characteristics of the school and the educational system -- e.g., available economic resources, the school's educational philosophy, and the quality of the teachers. Moreover, the school staff may provide students differential opportunities for self-direction, giving apparently brighter students greater opportunities to do self-directed work and apparently less bright students fewer such opportunities. Finally, educational self-direction is not entirely determined by the properties of the school and the differential treatment of the students. Even though opportunities for educational self-direction are to a substantial degree built into the structure of the school, there is still some leeway for similarly situated students to differ in how they respond to these opportunities. Students with higher levels of cognitive functioning may maximize whatever opportunity the school affords to do self-directed work; those with lower levels of cognitive functioning may avoid opportunities for self-direction. The hypothesis is that educational self-direction may not only affect, but also be affected by, cognitive functioning.

In empirically assessing this process, the lack of longitudinal data poses a serious problem: Ideational flexibility at earlier times cannot be statistically controlled in assessing the impact of educational self-direction on current ideational flexibility, nor can earlier educational self-direction be controlled in assessing the impact of ideational flexibility on current educational self-direction. There is, however, one great advantage in this analysis: There is information about the ideational flexibility of all of the fathers and most of the mothers. This means that, in assessing the impact of educational self-direction on the ideational flexibility of students, parental ideational flexibility can be statistically controlled. This makes it possible to statistically control genetic and, to some degree, family-experiential determinants of cognitive functioning. A model of the reciprocal effects of educational self-direction and ideational flexibility thus includes parental ideational flexibility, students' ages and grade levels, the extent to which school courses are compulsory or elective, and pertinent social characteristics of the students and their families.

The analysis strongly suggests that the degree of self-direction exercised by students in their schoolwork has a decided impact on their cognitive functioning and that their cognitive functioning, in turn, has a decided impact on their exercise of self-direction in schoolwork. Specifically, educational self-direction and ideational flexibility (the major aspect of cognitive functioning measured) have substantial, approximately equal, reciprocal effects. The impact of educational self-direction on ideational flexibility results mainly from the substantive complexity of students' schoolwork -- its scope, difficulty, and challenge. The effect of ideational flexibility on educational self-direction results in part from more intellectually flexible students exercising greater self-direction in their schoolwork, and in part from intellectually flexible students tending to be in somewhat higher grade

levels than are their age-mates -- and schoolwork is more self-directed at higher grade levels. Insofar as they can be tested, these conclusions apply to both secondary-school and college students.

The reciprocal effects of educational self-direction and ideational flexibility operate in context of other important processes. Thus, there are powerful effects of parental intellectual functioning and of student's age on students' ideational flexibility, and substantial effects of parental socio-economic status and students' grade levels on the amount of self-direction exercised in schoolwork. It is striking, though, that even in competition with these genetic, developmental, and social-structural factors, there is a substantial reciprocal relationship between the actual work that students do and their cognitive development.

This reciprocal process parallels what has previously been found for adults in the workplace. The present findings thus extend our knowledge of how work and personality affect one another in different institutional settings and at different stages of the life-course. In particular, they show that the experience of self-direction in work is important not only for the psychological functioning of adults, whose work is paid employment, but also for children and young adults, whose work is schooling. These findings also tell us something specific about education: They clearly weigh in on the side of those who argue that the "quality" of the school experience does make a difference. Moreover, they shed light on the question of what it is about schooling that affects students' cognitive development -- namely, the degree of self-direction they exercise in their schoolwork, particularly the substantive complexity of that schoolwork.

In addition to the analysis of educational self-direction and cognitive functioning, during this year an analysis of the relationship of educational self-direction to other aspects of psychological functioning -- in particular, self-directedness of orientation and psychological distress -- was also begun. Measurement models of self-directedness and distress analogous to those earlier developed for adult men and women were developed for the students, using confirmatory factor analysis. These models summarize several important aspects of personality. Self-directedness and distress are both "second-order" concepts. Self-directedness of orientation is indicated by personally responsible criteria of morality and by relatively low levels of authoritarian conservatism, fatalism, and idea conformity. Psychological distress is indicated by high levels of self-deprecation and anxiety, and by low levels of self confidence, trust, and idea conformity.

Preliminary causal models have been tested relating educational self-direction to self-directedness of orientation and to psychological distress. In the case of self-directedness of orientation, the investigators have found a pattern of reciprocal effects similar to (although not quite as statistically robust as) that for cognitive functioning; that is, educational self-direction and, particularly, the substantive complexity of schoolwork, leads to a more self-directed

orientation, and a more self-directed orientation leads to the exercise of greater self-direction in schoolwork. The investigators also found, as expected, negative reciprocal relationships between the exercise of educational self-direction and psychological distress. The substantive complexity of the schoolwork is again the major factor affecting psychological distress -- in this case, decreasing the student's level of distress. But closeness of supervision by teachers also plays a powerful role, increasing the distress of the student being supervised.

The investigators are in process of further testing these tentative results. During the coming year, they will also attempt to model the reciprocal effects between educational self-direction and students' values.

ASSESSING THE CORRELATION BETWEEN PARENTS' AND CHILDREN'S VALUES

In their current research, Kazimierz Slomczynski, Carrie Schoenbach, and Melvin Kohn are attempting, as a first step in the analysis of the process of value transmission, to reassess the magnitudes of the correlations between parents' and children's values. They are using data from representative samples of parents and children in the United States and Poland. To develop indices of values, they employ confirmatory factor analysis. Since confirmatory factor-analytic models enable one to separate the underlying constructs from errors in measurement of their indicators, such models provide a basis for calculating the "true" correlations between parents' and children's values, shorn of measurement error. They have developed a model for parents' and children's values in the United States and are in process of developing a similar model in Poland. In both countries, they focus on valuation of self-direction versus conformity to external authority. In these analyses, they build a single measurement model of mothers', fathers', and children's values, rather than three separate models. From these analyses, it appears that the correlations between parents' and children's valuation of self-direction/conformity are considerably stronger than past studies had led us to expect. For the United States, using longitudinal data and considering values for children across the entire age-range of 13 to 25 years of age, they find the correlation between fathers' and children's values to be approximately 0.58 and that between mothers and children to be approximately 0.55. Their analysis for Poland is still in process; it uses cross-sectional data and focuses on children in the younger half of that age-range. Preliminary indications are that the correlations for Poland will be not much lower than those for the United States -- probably just about the same magnitude as for U.S. children of the same age-range (when the U.S. data are analyzed cross-sectionally).

These correlations are much more in accord with everyone's a priori expectations than were those found in past studies -- a tribute, we believe, to the power of confirmatory factor analysis. But, still, even correlations of 0.58 and 0.55 leave much to be explained, with parents' values "accounting for" less than half of the variance in children's values. Moreover, as indicated in the provisional model of

value-transmission discussed above, calculating the correlations between parents' and children's values is only the first step in the causal analysis of the intergenerational transmission of values. The next step, on which the investigators will soon embark, is to begin to develop causal models that assess the degree to which social stratification affects children's values through its effects on parents' values (then transmitted from parents to children) and the degree to which social stratification affects children's values (if it does) through other processes. Social stratification aside, the questions of the extent to which and the processes by which values are transmitted from parents to children can now be dealt with on a much firmer basis than before, since we now have much more realistic estimates of the actual correlations between parents' and children's values.

It will also be possible in this research to assess the process of transmission of more general orientations than values, even to study the relationship between parents' and children's cognitive functioning. During this past year, indices of children's psychological functioning have been developed, in preparation for such an analysis.

THE POLISH REPLICATION

The main purpose of this inquiry has been to examine the interrelationship of social stratification, job conditions and psychological functioning in a socialist society. Three principal co-investigators, Kazimierz Slomczynski, Krystyna Janicka, and Jadwiga Koralewicz-Zebik, carried out in 1978 in Poland a precise replication of the survey originally conducted by Kohn and Schooler in 1964 in the United States. After the data had been collected, coded, and edited in Poland, Slomczynski brought them to NIH, where he, Joanne Miller, and Melvin Kohn have been analyzing them. Previous Annual Reports reviewed their development of methods designed to assure cross-national comparability of indices and their analysis of two of the central questions of the Polish replication: Do people's positions in the system of social stratification bear the same relationships to their values and orientations in socialist Poland as in the capitalist U.S.? If so, do these relationships result from the greater opportunities for occupational self-direction enjoyed by men of higher social-stratification position? As reviewed in detail in earlier Annual Reports, the answers to both questions are positive with respect to values and social orientations, but not with respect to self-conception.

Further comparative analysis of the Polish and U.S. data has taken three primary directions:

(1) The Polish investigators have carried out a new survey of the wives and children of a subsample of the men interviewed in the original study, with an emphasis on parent-child relationships and the psychological development of children. Slomczynski has been developing indices of family relationships and of various facets of mothers' and children's

psychological functioning, and he, Carrie Schoenbach, and Melvin Kohn are utilizing these data for their comparative analysis of value transmission (discussed above).

(2) Joanne Miller, Slomczynski, and Kohn have extended the comparative study of job conditions and psychological functioning in the United States and Poland to focus on age differentiation, examining variation in the determinants of personality for three groups of men: those 30 years or younger, those between the ages of 31 and 45, and those 46 years of age and older. This analysis is motivated by two central concerns: presumed changes in the malleability of personality across the life span and the possibility of differential importance of particular experiences depending on age. Differences across age groups might be expected because of biological aging, the proximity and duration of experience, or the social significance of experience depending on stage of career. The international comparison tests the universality of consistency or discrepancy across age groups.

This analysis examines four aspects of personality: intellectual flexibility, authoritarian conservatism, self-deprecation, and self-confidence. Several facets of psychological functioning are included, because of differences in their stabilities (intellective dimensions of personality being most stable and aspects of self-concept least stable) and because they may be differentially responsive to job conditions at different stages of career. For example, a highly stable characteristic such as intellectual flexibility may be more likely to be maintained by a fairly constant and accumulating set of experiences, regardless of age, than an aspect of personality normally fluctuating, such as self confidence. Moreover, the effects of particular job conditions may vary by age -- for example, the impact of close supervision may differ at different stages of career, perhaps especially so for self-esteem.

The analysis of age differentiation required extensive evaluation of indicators used to measure psychological functioning, establishing appropriate indices for each age group. When age-specific differences in indices occur, it is essential to statistically assess whether the indices nevertheless capture equivalent concepts. Otherwise, age-group comparisons could be interpreted as measurement artifacts. Although some age-specific differences in sets of patterned responses were identified in this study, these analyses indicate that it is possible to create conceptually equivalent indices of psychological functioning that are comparable across age groups.

The substantive emphasis of the study now focuses on the determinants of psychological functioning. The investigators are testing whether those conditions that facilitate or constrain opportunities for occupational self-direction have consistent effects across age groups. These analyses have not been completed. The initial findings for intellectual flexibility, though, are intriguing. Consistent with a general model of learning-generalization throughout the life course, occupational

self-direction is found to affect intellectual flexibility (independently of pertinent social characteristics), regardless of the age of the worker, in both Poland and the United States. Particularly striking is the consistency and strength of the effect of substantive complexity on intellectual flexibility. Future analysis of other dimensions of psychological functioning will specify the generalizability of these initial findings.

(3) Recently three kinds of analyses have been undertaken to evaluate the reliability of the measurement model of occupational self-direction in Poland. Krystyna Vanicka (of the Polish Academy of Sciences), and Grazyna Kacprowicz (University of Warsaw) and Slomczynski started by studying the effect of variation in procedures of data collecting used in interviewing the 1978 national Polish sample. The focus of analysis was on the effects of the interviewer's characteristics (e.g., level of field experience), modes of interviewing (e.g., number of probing questions), and methods of coding (e.g., extent of use of supplementary coding materials). None of these effects appeared to be statistically significant. Moreover, experiments of double and triple coding show the high reliability of each indicator of occupational self-direction, with intercoding correlations over .89, giving an average of .92.

A second kind of analysis has been based on data from a limited follow-up study. In this study, 100 men from the original 1978 national sample were interviewed for a second time with a schedule containing questions--repeated verbatim--that pertained to the substantive complexity of work. Two findings are of importance: the correlation between factor scores derived from the measurement model for the original and follow-up studies is very high ($r = .91$); and the hypothesis that measurement models for both studies are not based on the same sample is rejected at the .05 level of significance.

Third, the structure of the model developed for the 1978 Polish data has been imposed on two independent sets of data: the 1972 national sample and a 1979 Warsaw sample. In all three studies, the estimates of the model are essentially the same for the substantive complexity of work, the most important component of occupational self-direction. Estimates for closeness of supervision and routinization of work have been analyzed only for the 1978 and 1979 data, because only in these studies were the indicators obtained through the same method. The inter-study differences in the estimates appear to be negligible. Generally, the similarity of the measurement models of occupational self-direction across populations in Poland assures us that the reliability of the instrument for Poland is as good as for the United States.

THE JAPANESE REPLICATION

Another major replication of the Kohn-Schooler survey has been conducted in Japan by Atsushi Naoi and Ken'ichi Tominaga of the Department of Sociology of Tokyo University. Data collection took place during the

summer and fall of 1979. At that time, a probability sample of more than 800 employed men was interviewed with a questionnaire containing all the questions necessary for indexing job conditions and those aspects of psychological functioning measured in the original study. Data-analysis began in October, 1980, when Naoi came to the Laboratory as a Visiting Scientist to work collaboratively with Carmi Schooler. Confirmatory factor analysis was used to develop measurement models of occupational self-direction, intellectual flexibility, parental values and several facets of self-conception and social orientation. These measurement models proved to be generally similar to those that had previously been developed for the American sample. Last year's Annual Report provided a detailed description of the causal analyses that Naoi and Schooler did with these data -- analyses that generally confirm the U.S. findings.

This year's work on the study of the psychological effects of occupational conditions in Japan focused on the collection and preparation of data for further analysis. Michicko Naoi of the Tokyo Metropolitan Institute of Gerontology conducted a survey of the wives of the respondents in the earlier survey of Japanese men. Her questionnaire contains all of the key questions that we had asked the wives of our American respondents. We therefore now have the data to examine the generalizability to Japan of our American findings about the psychological effects on women of the conditions of both paid work and housework. In addition to the questions about values for themselves and their children, which were part of the American study, the Japanese women's questionnaire also contains a section on women's values and perceptions of their responsibilities vis-a-vis their own and their husbands' parents. These questions, when taken together with the questions the women were asked about the relative importance of various roles and about the value they place on their household work and paid employment, should permit the examination of the psychological effects of the web of obligations in which Japanese are said to be particularly enmeshed. These data have been coded and preliminary analyses begun.

Work also continues on the study of Japanese men. Data on jobs are being recoded to permit the examination of the effects of such theoretically important dimensions of work as whether it takes place in the primary or secondary sector of the economy and whether it derives from traditional Japanese culture. Other preparatory work has been the attempt to develop linear structural equation measurement models for parental values and values for self, which the investigators hope to use to examine the relationships between occupational conditions and values. Such analyses test the possibility that a single concept underlies a group of values and makes possible the purging of measurement error. Although it proved possible to develop a reasonable model for parental values, it did not appear possible to develop a satisfactory model of values for self.

THE PSYCHOLOGICAL CONSEQUENCES OF ENVIRONMENTAL COMPLEXITY

In the course of his work on this project, including his analyses of non-occupational determinants of psychological functioning, and

supplemented by extensive reading in related areas, Carmi Schooler has developed a more general interpretation of the effects of environmental complexity on psychological functioning. The consistency of the psychological effects of occupational self-direction in general, and of substantive complexity in particular, suggested a more general interpretation of the psychological consequences of complex environments and led to a broad review of studies of the effects of such environments throughout the life span.

According to this interpretation, the complexity of an individual's environment is defined by its stimulus and demand characteristics. The more diverse the stimuli, the greater the number of decisions required, the greater the number of considerations to be taken into account in making these decisions, and the more ill defined and apparently contradictory the contingencies, the more complex the environment. To the degree that the pattern of reinforcement within such an environment rewards cognitive effort, individuals should be motivated to develop their intellectual capacities and to generalize the resulting cognitive processes to other situations.

Non-intellective aspects of psychological functioning may also be affected by environmental complexity. To the extent that complex environments reward initiative and independent judgment, such environments should foster a generalized orientation favoring self-directedness rather than conformity to external authority. On the other hand, values, orientations and behaviors that are adaptive in complex environments may be maladaptive in simpler ones. Correspondingly, simple environments may not provide sufficient rewards to insure the development or continuance of relatively high levels of cognitive functioning and self-directedness. Consequently, continued exposure to relatively simple environments may result in a decrement in intellectual functioning and a change to values, orientations, and behaviors in keeping with the level of environmental demand. If, as seems plausible, such adaptation can occur at any age, changes in environmental complexity should produce changes in intellectual functioning and related values and orientations at any point in the individual's life course: midlife, childhood, old age.

In order to assess the plausibility of this interpretation evidence from a wide variety of sources was reviewed, including research about environmental effects on children and the aged, as well as animal and social psychological experiments. The review provides evidence that environmental complexity leads to more effective cognitive functioning across all stages of the life span. This effect has been found in both sexes, in several nations and even in species other than man. Although the evidence is not as extensive, the review also indicates that environmental complexity leads to a self-directed rather than conformist orientation. Although none of the studies is perfect, and few are able to completely exclude alternate explanations, it is rare to find a theory, no matter how roughhewn, that is congruent with such a range of phenomena--one which stretches not only across populations, but across the research of academic

disciplines from biochemistry to sociology.

Significance of the research:

This research is significant to the mission of the Institute on three distinct levels: (1) It has been well established that the incidence of schizophrenia is inversely related to social-stratification position. This relationship is not simply a function of greater genetic vulnerability at lower stratification levels or of more stressful life conditions at those levels. In larger part, it seems to result from people at lower stratification levels having less effective psychological mechanisms for coping with stress and uncertainty. This research, at a very basic level, is investigating what there is about the conditions of life associated with social-stratification position that results in people of lower social-stratification position having less effective mechanisms for coping with stress and uncertainty. (2) Above and beyond its interest in mental disorder, *per se*, the Institute has a mandate to study the conditions that facilitate and those that interfere with effective psychological functioning. This research has demonstrated that job conditions have appreciable effects on cognitive functioning, self-conceptions, and orientations to the outside world. Much of the recent work in this research project has focused on (a) demonstrating that job conditions actually do have a causal impact on effectiveness of psychological functioning and (b) elucidating the processes by which job affects psychological functioning. (3) As the research focuses more and more on the effects of social structure on the personality development of children, the potential relevance of what is learned here for programs of prevention of mental ill-health increases all the more, if only because some of the causal variables are particularly amenable to planned intervention.

Proposed course of further research:

As is evident above, the analysis of the Polish and Japanese replications is incomplete, with much more to be done. The analysis of education, too, is far from complete. In addition to completing these analyses, the investigators are now embarked on a new phase of the overall research program, an analysis of the processes by which parents' values and practices affect the values and personality development of their children. There are data in both the U.S. and Polish studies with which to carry out such an analysis. The investigators hope to elaborate and test the provisional model of these processes summarized in this Annual Report.

During this Fiscal Year, Melvin Kohn spent six weeks in Australia as a Visiting Fellow of the Research School of Social Sciences of the Australian National University, helping to develop (with Jonathan Kelley of that University) a parallel study of the transmission of values in Australian families. This study has potential value for extending the range of our analyses, since the values studied go far beyond the range of values included in our own research. Kohn also spent three weeks in the Federal Republic of Germany, part of that time as a member of the Scientific

Advisory Board of the Max Planck Institut fur Bildungsforschung, the rest of that time visiting and lecturing at German universities. Preliminary plans were made for possible future comparative studies that may further expand the scope of the Laboratory's research. (There have been several studies in Germany that replicate parts of our United States-based research. Others are in progress.)

Publications:

Kohn, M.L., and Schooler, C. (with the collaboration of Miller, J., Miller, K.A., Schoenbach, C., and Schoenberg, R.): Work and Personality: An Inquiry into the Impact of Social Stratification. Norwood, N.J.: Ablex Publishing Co., 1983.

Kohn, M.L., and Schooler, C.: Praca A Osobowosc: Studium Wspokzaleznosci. (Work and Personality: Study of their Interrelationships.) Warsaw: Polish Scientific Publishers (in press).

Kohn, M.L.: On the Transmission of Values in the Family: A Preliminary Formulation. In Kerckhoff, A.C. (Ed.), Research in Sociology of Education and Socialization: An Annual Compilation of Research. Greenwich, Connecticut: JAI Press, Vol. 4, 1983, pp. 1-12.

Kohn, M.L., and Schoenbach, C.: Social Stratification and Parental Values: A Multi-national Assessment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

Miller, K.A.: The Effects of Industrialization on Men's Attitudes Toward the Extended Family and Women's Rights: A Cross-National Study. J. Marriage Family. (In press)

Miller, K.A., and Kohn, M.L.: The Reciprocal Effects of Job Conditions and the Intellectuality of Leisure-Time Activity. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

Schooler, C.: Psychological and Social Perspectives on Status Attainment. Proceedings of the U.S.-Japan Conference on Social Stratification and Mobility. (In press)

Schooler, C.: The Application of Confirmatory Factor Analysis to Longitudinal Data. Pp. 155-171 in David Ricks and Barbara Dohrenwend (Eds.), Origins of Psychopathology: Research and Public Policy. Cambridge: Cambridge University Press, 1983.

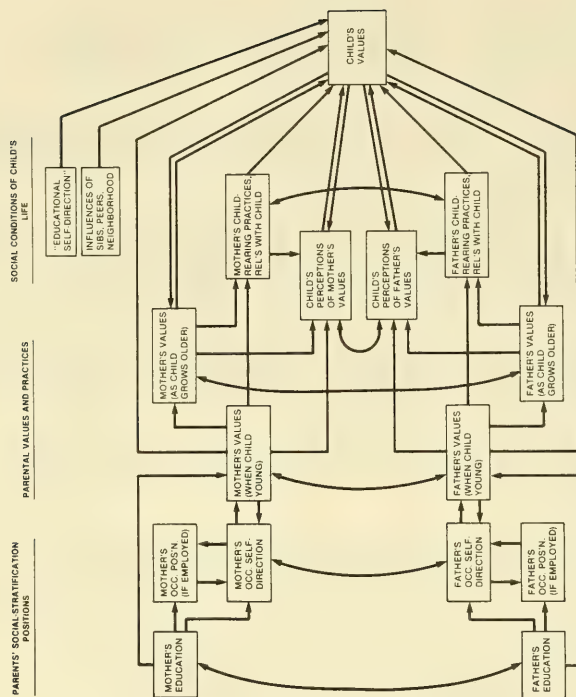


FIGURE 1. SCHEMATIC OUTLINE OF THE TRANSMISSION OF VALUES IN THE FAMILY.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00679-03 LSES

PERIOD COVERED

October 1, 1982, to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Structural Equation Models in the Analysis of Data with Measurement Error

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Ronald J. Schoenberg, Research Sociologist, LSES, NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Socio-environmental Studies

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this work is to further develop the methods and techniques for the specification and estimation of the parameters of structural equation models of survey data that contain random and nonrandom measurement error. Included in this are methods for the identification of the models, estimation of the means of unobserved variables, the determination of model condition, and the treatment of polytomous variables.

Project Description:

The key analytical instrument of research in the laboratory is a computer program written and maintained by Ronald Schoenberg called MILS (Multiple Indicator Linear Structural analysis). A new technique was added to the program this year which computes total and indirect effects and their standard errors. This has expanded considerably the analysis of causal relations among variables heretofore restricted just to direct effects. Now the effect of an independent variable on a dependent variable through other variables (i.e., an indirect effect) can be observed and tested, and thus a relationship between these variables can be established even though a direct effect may not exist.

During the year Schoenberg completed important work for the extension of latent variable methods to categorical and other limited variables--these are variables measured in categories or measured in some way that precludes all possible numerical values. The new technique generalizes and elaborates upon current methods for the analysis of limited variables such as logit, probit and Poisson regression. As a result these types of models may now be incorporated into multivariate measurement models such as that employed in MILS. Extensions of this work now in progress has important implications for data analysis methods used throughout the social science field.

In addition to personal consultation Schoenberg has kept the Laboratory up-to-date on research methods through a weekly one and a half hour seminar. Important recent developments in research methods such as log-linear modeling--the analysis of contingency tables--and event history analysis--the analysis of events using the mathematics of stochastic processes--have been covered in detail in these classes and are being applied to work in progress in the Laboratory.

Significance of the research:

The statistical methods and the computer program (MILS) that have been developed in the course of this project are fundamental to the research program of the Laboratory of Socio-environmental Studies, for they enable the investigators to deal straightforwardly with the two most important methodological problems faced in studying the effects of social structure

on personality: how to deal with measurement error and how to assess the direction of causal effects. These techniques are also proving to have considerable value to other Laboratories within the Intramural Research Program of NIMH and other Institutes of NIH and ADAMHA. Current work is designed to solve further statistical and methodological problems faced by intramural investigators and to make the computer program even more valuable.

Proposed course of further research:

An important goal for the coming year is the development of methods for the testing of the interaction effects of latent variables. This work will be crucial in all of the upcoming research in the Laboratory since nearly every aspect of this research will require the examination of potential interaction effects--interaction effects occur when a basic relationship between two variables changes as a function of a third variable.

Schoenberg will also continue his work on the analysis of categorical and other limited dependent variables.

Publications:

Parker, D.A., Parker, E.S., Schoenberg, R.J., and Brody, J.A.: Alcohol Use and Cognitive Loss among Employed Men and Women. Am. J. Public Health. 73: 521-526, 1983.

Schoenberg, R.J. and C. Richtand: An Application of the EM method to the Maximum Likelihood Estimation of Multiple Indicator and Factor Analysis Models. Sociol. Method Res. In press.

Schoenberg, R.J.: Statistical Models Must Be Appropriate: A Reply to Pat McGowan. Rev. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00680-01
PERIOD COVERED <u>October 1, 1982, to September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Work Experiences and the Deinstitutionalized Mentally Ill</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> <u>Elliot Liebow, Guest Worker, LSES, NIMH</u>		
COOPERATING UNITS (if any) <u>None</u>		
LAB/BRANCH <u>Laboratory of Socio-environmental Studies</u>		
SECTION 		
INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>.75</u>	PROFESSIONAL: <u>.75</u>	OTHER: <u>.75</u>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The objective of this <u>exploratory, participant observation</u> study is to examine the <u>work experiences</u> of the <u>deinstitutionalized mentally ill</u> over time and to seek out ways in which <u>job characteristics, symptoms, and social relationships</u> interact with one another to effect the course of recovery from <u>psychiatric disorder</u> and <u>reintegration into the community</u>. Field work is being carried out with residents of <u>halfway houses</u>, participants in <u>community-based psycho-social and transitional work programs</u>, and with <u>"unattached" deinstitutionalized men and women</u>. Data collection began in March 3, 1983, and is expected to end on December 31, 1983. </p>		

Project Description:

An exploratory, participant observer study of the relationship between work experiences and recovery from mental illness is being conducted by Elliot Liebow, on detail to the Laboratory from the Extramural program. Field work began in March of this year. This data collection phase is expected to last through this calendar year.

The major data base is to be derived from direct observation and personal interaction over time with men and women coming out of state mental hospitals, following them through their community integration experiences and focussing particularly on their work experiences and how these experiences affect the course of the disorder. The study population will be drawn from half-way houses, day treatment and vocational programs, and street people.

Specifically, the research focusses on (1) the way in which discrete aspects of the job (e.g., substantive complexity) interact with symptoms; (2) how job characteristics and symptoms interact with social relationships and social functioning; and (3) how all three systems--work, symptoms, social relationships--interact with one another to affect the course of recovery from psychiatric disorder.

The goal of this exploratory research is not to test hypotheses but rather (1) to grasp, so far as possible, the dynamics of the work-symptom-social relationship interactive processes that are the focus of this study, and (2) to use the hunches and insights that naturally flow out of first-hand, close-up observation over time, to generate hypotheses for a more systematic attack on the problem in the future.

Early first impressions include: (1) there is a lot of talk about the importance of work among service providers and their deinstitutionalized clients but surprisingly little action; (2) the routine programmatic emphasis on "entry level" jobs tends to ignore the wide range of individual skills, strengths and weaknesses of deinstitutionalized persons; and (3) many "entry level" jobs are among the most demanding, high-pressure jobs our society has to offer.

Significance of the research:

This project is directly pertinent to our understanding of rehabilitation of the deinstitutionalized mentally ill.

Proposed course of further research:

Data collection has barely begun. Most of the data collection and all other analysis has yet to be undertaken.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00424-08 LCB
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biologically Active Peptides in the Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Michael J. Brownstein, Chief, Laboratory of Cell Biology, NIMH		
COOPERATING UNITS (if any) U. Victoria, LDN/CH, NB/NIMH, St. Louis U., Salk Institute, State Univ. NY, U. Iowa, Mt. Sinai, USUHS, LNB/NIMH, U. Calif., San Diego, Johns Hopkins, LCS/NIMH, U. Chicago, NYU Med. School		
LAB/BRANCH Laboratory of Cell Biology		
SECTION Office of the Chief		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 10	PROFESSIONAL: 	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> We have continued to study the distribution of <u>peptide-containing cells</u> in the <u>central nervous system</u>, the biosynthesis of biologically active peptides, and the factors that regulate peptide <u>secretion</u>. Our studies of a number of peptides have contributed to a better understanding of the <u>cell</u> <u>biology</u> of peptidergic neurons and of their role in the brain. </p>		

Other Professional Personnel Engaged on Project

L. Eiden	LCB, NIMH
M. Palkovits	LCB, NIMH
M. Pruss	LCB, NIMH
E. Mezey	LCB, NIMH
V. Hook	LCB, NIMH
N. Zamir	LCB, NIMH
J. Russell	LCB, NIMH
J. Moskal	LCB, NIMH
R. Siegel	LCB, NIMH
N. Sherwood	U. Victoria
D. Klein	LDN, CH
A. Namboodiri	LDN, CH
H. Gainer	LDN, CH
P. Loh	LDN, CH
R. Eskay	NB, NIMH
M. Beinfeld	St. Louis University
W. Vale	The Salk Institute
J. Rivier	The Salk Institute
J. Speiss	The Salk Institute
P. Adams	State Univ. N.Y.
J. Kiss	U. Iowa
T. Williams	U. Iowa
D. Krieger	Mt. Sinai
H. Faden	USUHS
L. Skirboll	NB, NIMH
J. Baumgold	LNB, NIMH
I. Zimmerman	LNB, NIMH
R. Evans	The Salk Institute
M.G. Rosenfeld	U. California, San Diego
L. Fricker	Johns Hopkins
S. Snyder	Johns Hopkins
T. Reisine	LCS, NIMH
J. Axelrod	LCS, NIMH
F. Douglas	U. Chicago
T.T. Sun	NYU Medical School

Project Description

The structure of the salmon spawning factor (pGlu-His-Trp-Ser-Tyr-Gly-Trp-Leu-Pro-Gly-NH₂) has been determined by Drs. Sherwood, Eiden, Brownstein, Speiss, Rivier, and Vale. Dr. Sherwood has shown that a chromatographically similar peptide is present in the brains of a number of other fish of commercial import. She and her colleagues in Canada have shown that the teleost peptide is quite potent in inducing spawning in salmon, and Drs. Jones, Brownstein, Adams, and Rivier have shown that this molecule is also active on amphibian cells in vitro.

Drs. Sherwood, Eiden, and Brownstein have partially purified the LHRH from chicken brain. This peptide is different from the

teleost and mammalian forms of LHRH.

Drs. Brownstein, Namboodiri, and Klein have continued to work on purifying pineal serotonin N-acetyl-transferase.

Drs. Hook, Fricker, and Brownstein have prepared polyclonal and monoclonal antibodies against the carboxypeptidase B-like peptide processing enzyme from pituitary. These antibodies will be used for cell and molecular biological studies.

Dr. Hook has begun to characterize and purify the trypsin-like peptide precursor processing enzyme in adrenal medulla.

Drs. Mezey, Reisine, Palkovits, and Axelrod have implicated peripheral catecholamines in regulating ACTH release from the anterior pituitary. The amines stimulate ACTH secretion in vitro and in vivo by binding to β -adrenergic receptors on corticotrophs.

Drs. Mezey, Palkovits, Krieger, and Brownstein have shown by immunocytochemistry and by studying the effects of lesions on 5HT levels that 5HT in neuronal processes of the intermediate lobe of the pituitary is provided by neurons in the raphe complex and hypothalamic dorsomedial nucleus.

Drs. Mezey and Takahashi have localized G-protein to discrete populations of cells in the retina and central nervous system. Their light microscopic immunocytochemical studies are being extended to the EM level.

Dr. Moskal has succeeded in preparing monoclonal antibodies against neurons in dentate gyrus. These will be tested for behavioral and physiological effects.

Drs. Moskal, Baumgold, and Zimmerman have provided evidence for the existence of different molecular forms of the sodium channel protein in developing and adult rat brains.

Drs. Zamir, Palkovits, and Brownstein have examined the distribution of the dynorphin peptides (dynorphin A ₁₋₁₇, dynorphin A ₁₋₈, dynorphin B, α -neo-endorphin, β -neo-endorphin, Leu-enkephalin) in the rat brain. The data show that the dynorphin precursor may be processed differently in different terminals and suggest that this precursor is a major source of Leu-enkephalin in the brain. Dr. Zamir, et al., have also shown that the posterior pituitary is especially rich in dynorphin and that the source of most of this peptide is the supraoptic nucleus.

Dr. Russell has prepared monoclonal antibodies against calmodulin and calmodulin-related proteins; he is now preparing monoclonal antibodies against secretory granules from posterior

pituitary nerve endings. He hopes to use these in order to identify proteins important to the storage and release of peptide hormones.

Dr. Russell has identified two ATPases that are associated with secretory granules. One of these seems to be involved in axonal transport of these granules; the other with maintaining their ion gradients.

Drs. Mezey and Skirboll have identified a discrete population of cells in the hypothalamic paraventricular nucleus (PVN) that project to medullary catecholaminergic nuclei. They are attempting to determine the neurotransmitter used by the PVN cells by combining retrograde transport of dyes and immunocytochemistry.

Drs. Palkovits, Eskay, Eiden, Douglas, Williams, and Kiss have used immunocytochemistry and lesion/microassay methods to characterize a number of peptidergic pathways in the CNS. Special attention has been paid to the source of innervation of the median eminence.

Drs. Marangos, Brownstein, Evans, and Rosenfeld are in the process of isolating cDNA to neuron specific enolase (NSE) in RNA. This should allow the structure of NSE to be determined and the regulation of its biosynthesis to be studied.

Drs. Faden, Chiueh, Beinfeld, Zamir and Brownstein have examined the effect of TRH treatment on biochemical alterations that follow spinal cord trauma. While TRH protects against spastic quadriplegia, it does not seem to affect the loss of transmitters from descending neuronal pathways.

Dr. Eiden has examined the regulation of neuropeptide biosynthesis in cultured chromaffin cells. Radioimmunoassay and mRNA quantitation with complementary DNA probes allow determination of the locus of regulation (transcription, translation or post-translational processing) of enkephalin and VIP biosynthesis in these cells. Regulation of expression of both peptides seems to be mediated by intracellular cyclic AMP. A radioimmunoassay for chromogranin A has been developed, and we are presently engaged in cloning cDNA for the mRNA coding for chromogranin A. The regulation of biosynthesis of this major and apparently ubiquitous secretory protein will be compared to that of VIP and enkephalin.

Dr. Pruss has used tissue culture, immunohistochemistry and monoclonal antibodies to study secretory granule processing and neuropeptide synthesis and storage. She has prepared monoclonal antibodies to chromaffin granule membrane proteins and is studying membrane synthesis, compartmentation and processing using these antibodies as probes. In collaboration with Drs.

Hook, Eiden, and Hotchkiss, she is studying subcellular fractions of cultured chromaffin cells and factors which influence the expression of neuropeptides in these cells.

Dr. Siegel is investigating factors which are involved in regulating the level of different neuropeptides in cultured chromaffin cells and is utilizing tissue culture, immunohistochemistry and in situ hybridization in these studies.

Significance to Biomedical Research

Nerve cells use chemical "transmitters" to communicate with one another and with other target cells. Changes in transmitter biosynthesis, release, and/or metabolism have been suggested to result in nervous and mental disorders. Death of dopaminergic neurons in the substantia nigra, for example, is associated with the symptoms of Parkinson's disease. In the last ten years the number of putative neurotransmitters has increased by a factor of four or five. Most of the newly detected chemical messengers are peptides. Our knowledge of the anatomy, physiology and pharmacology of peptidergic neurons is comparatively incomplete at present; indeed, it is clear that many biologically active peptides remain to be isolated and characterized. The work outlined above is principally devoted to improving our understanding of peptide secreting nerve cells. To the extent that we understand these cells, we can formulate better hypotheses about their role in causing disease.

Proposed Course

The work outlined above is still in progress and will be continued.

Publications

Douglas, F.L., and Palkovits, M.: Distribution and quantitative measurements of somatostatin-like immunoreactivity in the lower brainstem of the rat. Brain Res. 242: 369-373, 1982.

Eiden, L.E., and Ruth, J.: Enkephalins modulate the responsiveness of rat atria in vitro to norepinephrine. Peptides 3: 475-478, 1982.

Eiden, L.E.: Recombinant DNA methods in neuroendocrinology: New answers to old questions. Peptides 3: 217-221, 1982.

Eiden, L.E., Loumaye, E., Sherwood, N., and Eskay, R.L.: Two chemically and immunologically distinct forms of luteinizing hormone-releasing hormone are differentially expressed in frog neural tissues. Peptides 3: 323-327, 1982.

Palkovits, M., Leranthy, Cs., Eiden, L.E., Rotsztein, W., and

Williams, T.H.: Intrinsic vasoactive intestinal polypeptide (VIP)-containing neurons in the baroreceptor nucleus of the solitary tract in rat. Brain Res. 244: 351-355, 1982.

Brownstein, M.J., Eskay, R.L., and Palkovits, M.: Thyrotropin releasing hormone in the median eminence is in processes of paraventricular nucleus neurons. Neuropeptides 2: 197-201, 1982.

Douglas, F.L., Palkovits, M., and Brownstein, M.J.: Regional distribution of substance P-like immunoreactivity in the lower brainstem of the rat. Brain Res. 245: 376-378, 1982.

Palkovits, M., Kiss, J.Z., Beinfeld, M.C., and Williams, T.H.: Cholecystikinin in the nucleus of the solitary tract of the rat: evidence for its vagal origin. Brain Res. 252: 386-390, 1982.

Palkovits, M., Brownstein, M.J., Eiden, L.E., Beinfeld, M.C., Russell, J., Arimura, A., and Szabo, S.: Selective depletion of somatostatin in rat brain by cysteamine. Brain Res. 240: 178-180, 1982.

Brownstein, M.J.: Post-translational processing of neuropeptide precursors. Trends Neurosci. 5: 318-320, 1982.

Sherwood, N., Eiden, L., Brownstein, M., Spiess, J., Rivier, J., and Vale, W.: Characterization of a teleost gonadotropin-releasing hormone. Proc. Natl. Acad. Sci. USA 80: 2794-2798, 1983.

Antoni, F.A., Palkovits, M., Makara, G.B., Linton, E.A., Lowry, P.J., and Kiss, J.Z.: Immunoreactive corticotropin-releasing hormone in the hypothalamoinfundibular tract. Neuroendocrinology 36: 415-423, 1983.

Brownstein, M.J.: Biosynthesis of vasopressin and oxytocin. Annu. Rev. Physiol. 45: 129-135, 1983.

Eiden, L.E., Eskay, R.L., Scott, J., Pollard, H., and Hotchkiss, A.J.: Primary cultures of bovine chromaffin cells synthesize and secrete vasoactive intestinal polypeptide (VIP). Life Sci. (in press), 1983.

Zamir, N., Palkovits, M., and Brownstein, M.J.: Distribution of immunoreactive dynorphin in the central nervous system of the rat. Brain Res. (in press), 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00422-12 LCB
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neuropharmacology of Circadian Rhythms		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Martin Zatz, Medical Officer (Research), Laboratory of Cell Biology, NIMH		
COOPERATING UNITS (if any) Department of Biology, University of Houston, Houston, Texas, Laboratory of Molecular Biology, University of Wisconsin, Madison, Wisconsin		
LAB/BRANCH Laboratory of Cell Biology		
SECTION Unit on Biochemical Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="margin-top: 20px;"> <p><u>Circadian rhythms</u> and environmental lighting regulate a number of endocrine and behavioral functions. <u>Cyclic GMP</u> mimics, and may mediate, the effects of light on the circadian oscillator in the <u>eye</u> of Aplysia. Monoclonal antibodies directed against rhodopsin permit visualization of photopigment in chick pineal, and differentiate among photopigment containing cells in chick and rat retina. Monoclonal antibody directed against the G protein regulating phosphodiesterase in retina also recognizes the G protein regulating adenylate cyclase in the rat pineal, indicating similarity (if not identity) of these proteins.</p> </div>		
(687)		

Other Professional Personnel Engaged on Project

J. Takahashi	LCB, NIMH
A. Eskin	Univ. of Houston
H. Hamm	Univ. of Wisc.
D. Bownds	Univ. of Wisc.
T. Reisine	LCS, NIMH
E. Mezey	LCB, NIMH

Project Description

Objectives: To elucidate the biochemical mechanisms and neuropharmacology of circadian rhythms.

Methods: Biochemical, pharmacologic, surgical, culture, radioimmunologic, and radioenzymatic techniques.

Major Findings: The avian pineal gland contains circadian oscillators and photoreceptors which regulate the synthesis of the hormone melatonin. Monoclonal antibodies produced against frog photoreceptor opsin were used to immunocytochemically visualize opsin-containing cells in the chick pineal. Specific opsin staining was localized in outer-segment-like processes in a cell type that lines the lumen of cellular rosettes or follicles distributed throughout the gland.

Characterization of the rhodopsin monoclonal antibodies in retinal tissue strongly suggests that five of the clones tested produce opsin antibodies that are specific to rod photoreceptors in chicken and rat. Antibodies from another clone appeared to label the inner segment of selected cone photoreceptors in chicken retina. Unlike previous polyclonal opsin antibodies, these monoclonals appear to distinguish between rod and cone visual pigments.

A monoclonal antibody against frog photoreceptor "transducin" (a guanine nucleotide regulatory protein that activates a light-sensitive phosphodiesterase in rods) was found to inhibit hormone and guanine nucleotide stimulation of adenylate cyclase activity in rat pineal membranes. The antibody was able to immunoprecipitate ³²P-labeled ADP-ribosylated proteins catalyzed by cholera toxin. These experiments strongly suggest that structural homologies exist among a family of GTP-binding proteins that regulate cyclic nucleotide metabolism.

The eye of Aplysia contains a circadian pacemaker that can be studied in vitro. We have found that light increases intracellular cyclic GMP levels by 50%. To test whether the increase in cyclic GMP is involved in conveying light information to the circadian oscillator, the effect of 6-hour treatments with 8-bromo cyclic GMP on the phase of the circadian rhythm was investigated. Treatment with the cyclic GMP analogue completely

mimicked the phase-shifting effect of light: the phase response curves for light and for 8-bromo cyclic GMP were identical. The cyclic GMP-induced phase shift could be blocked by low sodium treatment, suggesting that a sodium-dependent membrane potential change (required for the effects of light) is also required for the cyclic GMP effect. Previously we showed that cyclic AMP mediated the effects of a second input to the oscillator, serotonin. It is intriguing that these two inputs to the clock, light and serotonin, appear to be mediated at the intracellular level by two different cyclic nucleotide "second messengers".

Significance to Biomedical Research: Circadian rhythms occur in hormone levels, activity, mood, temperature, and other physiologic functions. Elucidation of the mechanisms generating and regulating circadian rhythms are of broad clinical and biologic interest.

Proposed Course of Project: Mechanisms generating and regulating circadian rhythms and the effects of light will be explored further.

Publications

Takahashi, J.S., and Zatz, M.: Regulation of circadian rhythmicity. Science 217: 1104-1111, 1982.

Eskin, A., and Takahashi, J.S.: Adenylate cyclase activation shifts the phase of a circadian pacemaker. Science 220: 82-84, 1983.

Zatz, M.,: The pineal gland. In Kebabian, J.W., and Nathanson, J.A.(Eds.): Handbook of Experimental Pharmacology. New York, Springer-Verlag, 1983, Vol.58/II, pp.691-710.

Nestler, E.J., Zatz, M., and Greengard, P.: A diurnal rhythm in pineal protein I content mediated by beta-adrenergic neurotransmission. Science 217: 357-359, 1982.

Latker, C.H., Eiden, L.E., and Zatz, M.: The effect of methylazoxymethanol acetate (MAM) on the developing rat retina. Exp. Eye Res. 35: 351-361, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00429-04-LCB
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemistry of Membranes		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Martin Zatz, Medical Officer (Research), Laboratory of Cell Biology, NIMH		
COOPERATING UNITS (if any) NPM/NICHHD, LVR/NEI		
LAB/BRANCH Laboratory of Cell Biology		
SECTION Unit on Biochemical Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.8	PROFESSIONAL: 1.6	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Incubation of <u>membranes</u> with <u>S-adenosylmethionine</u>, <u>acyl coenzyme A</u>, and <u>FeSO₄</u> results in the formation of S-methyl-acylthioester. This is a novel reaction which could participate in drug metabolism.</p> <p><u>Pantethine</u>, a metabolic precursor for <u>coenzyme A</u>, depletes cystinotic cells of <u>cystine</u>. This could provide a safe, effective <u>treatment for cystinosis</u>.</p> <p>Bovine <u>retina</u> incorporates <u>palmitate</u> into <u>rhodopsin</u>.</p>		

Other Professional Personnel Engaged on Project

S. Markey	LCB,	NIMH
S. Engelsen	LCS,	NIMH
J. DeB. Butler	NPM	NICHHD
P. O'Brien	LVR,	NEI

Project Description

Objectives: a) to identify the nonpolar lipids formed from S-adenosylmethionine; b) to explore the physiology and pharmacology of novel products of coenzyme A metabolism; c) to explore the role of lipid metabolism in the regulation of rhodopsin biosynthesis and function.

Methods: Biochemical, chromatographic, chemical, pharmacologic, tissue culture, and radioactive trace techniques.

Major Findings: a) Incubation of membranes from a number of organs with radioactive S-adenosylmethionine and oleoylcoenzyme A results in the formation of S-methyl-oleoylcysteamide. Addition of FeSO_4 , but not other divalent cations, to the reaction mixture resulted in the formation of a previously unidentified lipid which was identified as S-methyl-acylthioester by mass spectrometry after isolation by extraction, HPLC, and GC. Formation of this product involves an enzymatic process, which can occur on the cytoplasmic side of plasma membranes. Synthesis of these S-methylacylthioesters is novel in that neither acyl-CoA nor S-adenosylmethionine would be expected to provide precursors for such products. The properties of these thioesters are such that, if formed in vivo, they could act as methylthio donors. b) One of the minor products of coenzyme A metabolism is cysteamine. This substance, which is related to the amino acid cystine, is effective in lowering the excessively high levels of cystine in cells from patients with the genetic disease cystinosis. We have found that pantethine, a metabolic precursor of both coenzyme A and cysteamine, is as effective as cysteamine in depleting cystinotic cells of cystine. Pantethine's mechanism of action involves its intracellular conversion to pantothenic acid and cysteamine. Cysteamine then removes cystine from the cells via efflux of the mixed disulfide cysteinyl cysteamine. c) Recent reports indicate that fatty acyl groups may be covalently attached to intrinsic membrane glycoproteins during their biosynthesis. Rhodopsin is an intrinsic membrane glycoprotein which mediates photoreception. We have found that bovine retinas incubated with radioactive palmitate incorporate radioactivity which remains attached to rhodopsin through organic extraction, affinity chromatography, agarose chromatography, bleaching, and SDS gel electrophoresis. Base hydrolysis releases free fatty acid and hydroxylamine releases the hydroxamate, suggesting a covalent ester bond. Subcellular fractions are also capable of incorporating palmitate into rhodopsin and further

experiments are required to determine whether the reaction is enzymatic.

Significance to Biomedical Research: a) Investigators in drug metabolism have recently reported the in vivo formation of methylthio derivatives of xenobiotics, carcinogens, and drugs. S-Methylacylthioesters could provide the source of these methylthio groups, whose addition is likely to modify the action of drugs. b) Systemic cysteamine is poorly tolerated by patients with cystinosis. Pantethine, a natural dietary constituent, may provide a safe, effective, and preferable alternative to cysteamine for the treatment of cystinosis. c) The role of membrane lipids in cellular function, particularly in signal transduction, is currently being recognized and elucidated. Rhodopsin is a prototypical membrane transducer; elucidation of its interaction with lipids is of specific and general importance for understanding neuronal membranes.

Proposed Course of Project: a) Completed. b) The mechanism of action of pantethine and cystamine on cystinotic cells and its relationship to the underlying defect will be investigated. c) Interaction of photoreceptor membranes with specific lipids will be investigated.

Publications

Engelsen, S.J., and Zatz, M.: Stimulation of fatty acid methylation in human red cell membranes by phospholipase A₂ activation. Biochim. Biophys. Acta 711: 515-520, 1982.

Zatz, M., Engelsen, S.J., Kloog, Y., Dudley, P.A., and Markey, S.P.: Methylation of nonpolar lipids: Identification and characterization. In Borchardt, R.T., and Creveling, E. (Eds.): Biochemistry of S-adenosylmethionine and related compounds. London, Macmillan, 1982, pp. 509-512.

Zatz, M., Engelsen, S.J., and Markey, S.P.: Biosynthesis of S-methyl-N-oleoylmercaptoethylamide from oleoyl coenzyme A and S-adenosylmethionine. J. Biol. Chem. 257: 13673-13678, 1982.

Bougnoux, P., Bonvini, E., Stevenson, H.C., Markey, S.P., Zatz, M., and Hoffman, T.: Identification of ubiquinone-50 as the major methylated nonpolar lipid in human monocytes. Regulation of its biosynthesis via methionine dependent pathways and relationship to superoxide production. J. Biol. Chem. 258: 4339-4344, 1983.

Zatz, M., Engelsen, S.J., and Markey, S.P.: Novel formation of S-methyl-acylthioester from oleoyl coenzyme A and S-adenosylmethionine in the presence of FeSO₄. J. Biol. Chem. 258: 5759-5763, 1983.

Butler, J. DeB., and Zatz, M.: Pantethine depletes cystinotic fibroblasts of cystine. J. Pediatr. 102: 796-798, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00427-06 LCB
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) On the Mechanism of Signal Transduction Through Receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Fusao Hirata, Visiting Scientist, Laboratory of Cell Biology, NIMH		
COOPERATING UNITS (if any) LCS/NIMH, LCM and LBG/NHLBI, LDBA and LMI/NIDR, LID/NCI, Johns Hopkins University		
LAB/BRANCH Laboratory of Cell Biology		
SECTION Unit on Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">5.0</div>	PROFESSIONAL: <div style="text-align: center;">5.0</div>	OTHER: <div style="text-align: center;">0.0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A receptor-mediated cascade of phospholipid metabolism was analyzed. This cascade involves (a) interaction of receptors with specific ligands, (b) increased <u>phospholipid methylation</u>, (c) increased flux of Ca^{2+}, (d) activation of kinases, (e) phosphorylation of <u>lipomodulin</u>, a phospholipase inhibitory protein, (f) activation of <u>phospholipases</u>, (g) release of arachidonic acid and its metabolites such as prostaglandins and leukotrienes and (h) increased uptake of glucose. Lipomodulin was purified from culture media of HL60 cells and monoclonal antibodies against purified protein were isolated. This protein appeared to be associated with <u>the HLA gene</u> and was suggested to play an important role in cell-cell interactions. </p>		

Other Professional Personnel Engaged on Project

Y. Notsu	LCB, NIMH
K. Matsuda	LCB, NIMH
T. Matsumoto	LCB, NIMH
C. Pezzoli	LCB, NIMH
T. Hattori	LCB, NIMH
R. Herberman	LID, NCI
J. Axelrod	LCS, NIMH
E. Schiffman	LDBA, NIDR
R. Siraganian	LMI, NIDR
M. Vaughan	LCM, NHLBI
M. Nirenberg	LBG, NHLBI
D. Newcomb	Johns Hopkins
K. Ishizaka	Johns Hopkins

Project Description

Objectives: To study the mechanism of signal transduction through receptors including regulation of ion fluxes, cyclic nucleotide production, and glucose metabolism.

Methods: Enzymatic, radiometric, immunological, pharmacological and molecular biological techniques.

Major Findings: Dr. Hirata and his colleagues have previously shown that arachidonic acid, a precursor of prostaglandins and leukotrienes, is released from membrane phospholipids after stimulation of various receptors. The arachidonic acid is derived from methylated phospholipids (phosphatidylcholine and/or phosphatidylinositol), suggesting that the activation of phospholipases has a key role in its production. The activities of phospholipases in a variety of cells were suggested to be controlled by phosphorylation-dephosphorylation of lipomodulin. Lipomodulin was isolated initially from rabbit neutrophils treated with glucocorticoids and recently from HL60, human promyeloleukemia cells. The isolated protein has glucocorticoid-like activities such as promotion of cellular differentiation, suppression of immunoglobulin synthesis and inhibition of chemotaxis. All these activities have been attributed to this protein's inhibition of phospholipases.

Significance to Biomedical Research: Glucocorticoids are hormones secreted by the adrenal cortex. They have a variety of biological effects including anti-inflammation, anti-edema (including brain edema) and anti-cancer activities. Lipomodulin can mimic most of these activities, suggesting that it may prove a useful diagnostic and therapeutic agent.

Proposed Course of Project: The amino acid composition and sequence of lipomodulin will be determined and cDNA to

lipomodulin mRNA will be cloned. The lipomodulin gene will subsequently be characterized. Lipomodulin levels in plasma of patients with various diseases such as depression, psoriasis, and allergy will be measured by a recently developed radioimmunoassay to correlate lipomodulin levels with disease states.

Publications

Daëron, M., Sterk, A.R., Hirata, F., and Ishizaka, T.: Biochemical analysis of glucocorticoid-induced inhibition of IgE-mediated histamine release from mouse mast cells. J. Immunol. 129: 1212-1218, 1982.

Hirata, F., Notsu, T., Iwata, M., Parente, L., Dirosa, M., and Flower, R.J.: Identification of several species of phospholipase inhibitory protein(s) by radioimmunoassay for lipomodulin. Biochem. Biophys. Res. Commun. 109: 223-230, 1982.

Shitara, N., McKeever, P.E., Cummins, C., Smith, B.H., Kornblith, P.L., and Hirata, F.: Beta-adrenergic receptor desensitization stimulates glucose uptake in C₆ rat glioma cells. Biochem. Biophys. Res. Commun. 109: 753-761, 1982.

Steinberg, A.D., Smolen, J.S., Sakane, T., Kumagai, S., Morimoto, C., Chused, T.M., Green, I., Hirata, F., Siminovitch, K.A., and Steinberg, R.T.: Immune regulatory abnormalities in systemic lupus erythematosus. In Cummins, Michael, and Wilson (Eds.): Immune Mechanisms in Renal Disease. New York, Plenum, 1982, pp. 529-548.

Bareis, D.L., Manganiello, V.C., Hirata, F., Vaughan, M., and Axelrod, J.: Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation and CycAMP accumulation in human fibroblasts. Proc. Natl. Acad. Sci. USA 80: 2514-2518. 1983.

Crews, F.T., Camacho, A., Phillips, I., Calderini, G., Hirata, F., Axelrod, J., McGivney, A., and Siraganian, R.P.: Effects of membrane fluidity on mast cell and nerve cell function. In Horrocks, L.(Ed.): Phospholipids in the Nervous System. New York, Raven Press, 1982, Vol. 1, pp. 21-35.

Hattori, T., Hoffman, T., and Hirata, F.: Differentiation of a histocytic lymphoma cell line by lipomodulin, a phospholipase inhibitory protein. Biochem. Biophys. Res. Commun. 111: 551-559, 1983.

Hattori, T., Hirata, F., Hoffman, T., Hizuta, A., and Herberman, R.B.: Inhibition of human natural killer (NK) activity and antibody dependent cellular cytotoxicity (ADCC) by lipomodulin, a phospholipase inhibitory protein. J. Immunol. (in press), 1983.

Hirata, F.: Lipomodulin: A possible mediator glucocorticoids' action. In Samuelson, R., Paoletti, R., and Ramwell, P. (Eds.): Advances in Prostaglandin Thromboxane and Leucotrien Research. New York, Raven (in press), 1983.

Hirata, F.: Lipomodulin and its regulation of cellular phospholipase(s). In Proc. of French Society of Rheumatology. (In press), 1983.

Hirata, F.: Role of lipomodulin, a phospholipase inhibitory protein in immunoregulation. In Advances in Inflammation Research. New York, Raven (in press), 1983.

Hirata, F.: Drugs that inhibit the activities or activation of phospholipases and other acylhydrolases. In Handbook of Prostaglandins. CRC Press (in press), 1983.

Hirata, F.: Phospholipid methylation. In Lajtha, A. (Ed.): Handbook of Neurochemistry. New York, Raven (in press), 1983.

Hirata, F., and Iwata, M.: Role of lipomodulin, a phospholipase inhibitory protein, in immunoregulation by thymocytes. J. Immunol. 130: 1930-1936, 1983.

Schiffmann, E., Geetha, V., Pencev, D., Warabi, H., Mato, J., Hirata, F., Brownstein, M., Manjunath, R., Mukgerjee, A., Liotta, S., and Terranova, V.P.: Adherence and regulation of leukotaxis. In 1st International Symposium on Chemotaxis and Inflammation, Genova, Switzerland (in press), 1983.

Uede, T., Hirata, F., Hirashima, M., and Ishizaka, K.: Modulation of the biologic activities of IgE-binding factors. I. Identification of glycosylation inhibiting factor as a fragment of lipomodulin. J. Immunol. 130: 878-884, 1983.

Axelrod, J., and Hirata, F.: Phospholipid methylation and the receptor induced release of histamine from cells. Trends in Pharmacol. Sci. 3: 156-158, 1982.

Bareis, D.L., Hirata, F., Axelrod, J., and Schiffman, E.: Phospholipid metabolism, calcium flux and the receptor mediated induction of chemotaxis in rabbit neutrophils. J. Cell Biol. 93: 690-697, 1982.

Hirata, F.: Overview on phospholipid methylation. In Usdin, E., Borchart, R. Creveling, C. (Eds.): Biochemistry of S-adenosylmethionine and Related Compounds. New York, MacMillan Press, 1982, pp. 109-117.

Hirata, F., and Axelrod, J.: Biochemical mechanism of signal transduction across biomembranes. In Yoshida, H., Yamamura, H.I.

(Eds.): Neurotransmitter Receptors. New York, John Wiley & Sons, Inc., 1982, pp. 287-294.

Hirata, F., Crews, F.T., Axelrod, J., McGivney, A., Siraganian, R.P., Ishizaka, T., and Ishizaka, K.: Biochemical mechanism of histamine release from rat peritoneal mast cells and rat basophilic leukemia cells. Adv. Biosci. 33: 43-45, 1982.

Siraganian, R.P., McGivney, A., Barsumian, E.L., Crews, F.T., Hirata, F., and Axelrod, J.: Use of variants of the rat basophilic leukemia cell line for study of histamine release. Fed. Proc. 41: 30-34, 1982.

Toyoshima, S., Hirata, F., Axelrod, J., Beppu, M., Osawa, T., and Waxdal, M.J.: Relationship between phospholipid methylation and calcium influx in murine lymphocytes stimulated with native and modified concanavalin A. Mol. Immunol. 19: 229-234, 1982.

Toyoshima, S., Iwata, M., Hirata, F., Axelrod, J., Osawa, T., and Waxdal, M.J.: Phospholipid methylation: a possible role in lymphocyte mitogenesis. Mol. Immunol. 19: 467-476, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01836-05 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (30 characters or less. Title must fit on one line between the borders.) Receptors in the Central Nervous System: Biochemistry to Behavior		Benzodiazepine
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Steven M. Paul, Chief, Clinical Neuroscience Branch, NIMH		
COOPERATING UNITS (if any) Laboratory on Bioorganic Chemistry, NIADDK; Clinical Psychobiology Branch, NIMH; Section on Brain Biochemistry, NSB, NIMH; Clinical Neuropharmacology Branch, NIMH, Section on Molecular Pharmacology, NS, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Preclinical Studies		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.9	PROFESSIONAL: 4.3	OTHER: 0.6
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) High affinity and stereospecific receptors for benzodiazepines have been identified in the mammalian central nervous system. It is currently believed that the interaction of benzodiazepines with their receptors initiates a series of neuronal events resulting in an <u>enhancement of GABA-mediated chloride permeability</u> . The latter results <u>behaviorally</u> in the major pharmacological actions of benzodiazepines, namely their anxiolytic, anticonvulsant, hypnotic, and muscle relaxant actions. In addition to benzodiazepines, a variety of sedative/hypnotic agents of the minor tranquilizer class appears to interact with one or more components of the benzodiazepine/GABA receptor complex, and thus the latter has been proposed as a <u>common site of minor tranquilizer action</u> . Several aspects of the benzodiazepine/GABA receptor complex are currently being studied, including <u>purification of the receptor by affinity chromatography</u> , characterization of <u>multiple binding sites</u> on the receptor complex which recognizes agonists, antagonists or inverse agonists, strain and genetic differences in receptor and number and conformation, the behavioral and biochemical effects of novel (non-benzodiazepine) anxiolytics as well as "anxiogenic" inverse agonists, and the identification of a novel benzodiazepine receptor in the CNS for <u>4-chlorodiazepam (Ro5-4864)</u> , the so-called peripheral benzodiazepine receptor ligand.		

Other Professional Personnel:

Phil Skolnick	Pharmacologist	LBC NIADDK
Philip T. Ninan	Clinical Associate	NS NIMH
Aaron J. Janowsky	Guest Worker	PRAT NIGMS
Jacqueline N. Crawley	Res. Biologist	NS NIMH
Daniel Hommer	Staff Psychiatrist	NS NIMH
Wallace Mendelson	Staff Psychiatrist	CP NIMH
Thomas Insel	Staff Psychiatrist	CN NIMH
Ellis Kempner	Physicist	LPB NIADDK
John Glowa	Guest Worker	NS NIMH
Remie Quirion	Guest Worker	NS NIMH
Rochelle Schwartz	Pharmacologist	NS NIMH
John Thomas	Chemist	NS NIMH
G. Chrousos	Staff Physician	DNB NICHHD
James Cook	Chemist	NICHHD, Univ. of Wisconsin
Margaret M. Schweni	Staff Fellow	LPB NIADDK
Gordon T. Bolger	Visiting Fellow	LBC NIADDK
Ben Avi Weissman	Visiting Scientist	LBC NIADDK
Karl H. Weber	Guest Worker	LBC NIADDK

Project Description:Methods Employed:

Radioreceptor techniques have been employed in the neurochemical characterization of benzodiazepine receptors and as a means of detecting and quantitating endogenous substances which may regulate these sites. Other biochemical techniques employed include radioenzymatic assay, thin layer, ion exchange and high pressure liquid chromatography, gel filtration and molecular size exclusion chromatography. Pharmacologic testing includes quantitation of the sensitivity of mice to chemical convulsants. The convulsant action of various compounds has been monitored in rats using electroencephalographic techniques combined with fast Fourier analysis. Electroencephalography has also been used to detect sleep stages in the rat. Ataxia and muscle relaxation have been tested through the use of rotating rod and "wire-grip" procedures. Anxiolytic (anticonflict) action has been examined using either a mouse model of behavior (which measures the activity of mice in a novel environment) or alternatively, using a rat "conflict" model of behavior (the thirsty rat conflict test). Blood pressure in rodents has been measured using an indirect (tail cuff) technique. Anxiolytic/anxiogenic behavior in primates is currently being investigated using behavioral rating scales (modified Redmond) as well as measuring the somatic and endocrine markers of anxiety (pulse rate, mean arterial blood pressure, and plasma catecholamines, ACTH, β -endorphin and cortisol).

Major Findings:

Previous studies from this laboratory have demonstrated that recognition sites (receptors) for benzodiazepines are functionally linked to receptors for gamma-aminobutyric acid (GABA) (the major inhibitory neurotransmitter in mammalian C.N.S.) and an associated chloride channel. We also demonstrated that certain C-3 substituted β -carboline which bind to benzodiazepine receptors can antagonize the major pharmacologic actions of the benzodiazepines. Furthermore, the

"regulatory" properties of the benzodiazepine receptor (that is, the apparent changes in receptor affinity observed in the presence of pharmacologic agents) are markedly different when the receptor is occupied by "benzodiazepine antagonists" or "benzodiazepine-like" (agonist) compounds. These findings led to the development of a simple and sensitive *in vitro* test to differentiate benzodiazepine "agonists" and "antagonists," and supported earlier electrophysiological and pharmacological observations demonstrating the importance of GABA in mediating the therapeutic actions of the benzodiazepines.

The apparent affinities of a novel series of "annelated" heterocyclic benzodiazepines has been examined in the presence and absence of GABA. These compounds have anxiolytic and anticonvulsant activity. Some of these derivatives lack hypnotic actions. The apparent affinity of these compounds, in contrast to other "benzodiazepine-like" drugs, was not significantly altered by GABA. Furthermore, in contrast to other compounds (both agonists and antagonists) which interact with benzodiazepine receptors *in vitro*, the apparent affinities of these compounds did not appear to be temperature dependent between 0-37°C. These findings may invalidate the utility of the *in vitro* test developed in this laboratory, since "false negatives" have now been found. These findings also imply that GABA may not be required for all the pharmacological actions of the benzodiazepines and that the "state-transitions" theory of agonist/antagonist occupation of receptors may be invalid.

Marked strain differences to the convulsant actions of 3-carbomethoxy- β -carboline (β -CCM) have been observed in mice. This difference was initially believed to present an important model to study the neurochemical bases by which a compound binding to the benzodiazepine receptor could exert actions best described as pharmacologically "opposite" to the benzodiazepines. Further investigation revealed no differences in the neurochemical characteristics of the benzodiazepine receptor in the two strains. However, significant differences in brain levels of β -CCM were found in the two strains. These data suggest that pharmacokinetic, rather than pharmacodynamic factors account for the marked strain differences in susceptibility observed to the convulsant actions of β -CCM.

The benzodiazepine antagonist, 3-carboethoxy- β -carboline (β -CCE) had been reported not to elicit seizures, but rather possessed benzodiazepine antagonist and proconvulsant actions. The observation that the binding of [^3H] β -CCM and DMCM (two closely related derivatives of β -CCE with convulsant actions) was significantly inhibited by GABA while the binding of [^3H] β -CCE was not, led to the hypothesis that GABAergic inhibition by these compounds might be responsible for their convulsant actions. However, we have shown that the $t_{1/2}$ of β -CCE is extremely short in rodent plasma ($t_{1/2}$ 0.4 min), while β -CCM is degraded 3-4 fold more slowly, and DMCM not at all. This observation led to an electroencephalographic study of the effects of intravenously administered β -CCE in rats. This study demonstrated that β -CCE could elicit electroencephalographic seizures in rats, which are blocked by the benzodiazepine antagonist, CGS-8216. β -CCE was observed to be a potent convulsant in squirrel monkeys following intramuscular administration. This observation led to a detailed pharmacokinetic and pharmacodynamic study of β -CCE in these species. It was observed that in contrast to rat, β -CCE is degraded very slowly in monkey ($t_{1/2}$ > 25 min). Furthermore, no significant differences in the "coupling" of [^3H] β -CCE binding were observed in rat and monkey. These observations

suggest that pharmacokinetic factors play an important role in determining the pharmacologic profile of a series of pharmacologically important β -carboline esters.

It was previously demonstrated that anxiolytic barbiturates such as pentobarbital enhance the binding of [3 H] benzodiazepines to benzodiazepine receptors in vitro. This has been proposed to be the mechanism by which such compounds exert their anxiolytic actions. It has now been shown that the benzodiazepine antagonist CGS-8216 can block the anxiolytic actions of pentobarbital at doses which by itself does not affect "conflict" responding. However, at higher doses, CGS-8216 by itself reduces punished responding of rats, suggesting that CGS-8216 may be "anxiogenic" at these doses, and that the modified Vogel test employed (i.e. with a lower level of punishment) may be used to detect anxiogenic as well as anxiolytic actions of drugs in rodents.

Administration of β -CCE to chair adapted rhesus monkeys resulted in a syndrome reminiscent of anxiety or fear in humans. In addition to marked behavioral changes, somatic (e.g., increase in heart rate and blood pressure) and endocrine (e.g. robust increases in plasma ACTH, β -endorphin, cortisol, and catecholamines), changes were also observed at doses as low as 100 μ g/kg. Remarkably, when the subject was approached by the rater following administration of low doses (100-200 μ g/kg) of β -CCE, the same degree of rater interaction which elicited no response in vehicle-treated animals elicited large changes in the behavioral, somatic, and endocrine syndrome which mimicked that seen in animals administered larger (> 1 mg/kg) doses of β -CCE. These results suggest that β -CCE elicits a behavioral "sensitization" of the animals, so that a non-threatening situation may now be perceived as "anxiety-producing." These findings strongly suggest that the benzodiazepine receptor is not only involved in the anxiolytic actions of the benzodiazepines but also in the physiologic generation of anxiety. Beta-CCE-induced alterations in behavior are blocked by the benzodiazepine, diazepam, as well as the benzodiazepine antagonist Ro 15-1788. Drugs which have been reported to possess anxiolytic actions, such as the β -adrenoceptor blocker propranolol and the α -adrenoceptor blocker, clonidine, have also been examined in this model. These compounds have clinical potency as anxiolytics but have not been demonstrated to directly affect the benzodiazepine-GABA receptor chloride ionophore complex. Propranolol completely reversed the increase in heart rate elicited by β -CCE but did not significantly reduce the behavioral activation, blood pressure changes, or increases in plasma cortisol elicited by β -CCE. In some cases these were actually potentiated by propranolol. Clonidine, at a dose which by itself reduced heart rate and blood pressure also antagonized β -CCE elicited increases in both these parameters but only partially blunted the behavioral actions. These observations suggest both clonidine and propranolol may exert their anxiolytic actions through a peripheral, rather than a central mechanism. These experiments also demonstrate the potential use of this model as a means of analyzing the mechanism of action of anxiolytic agents.

Although the benzodiazepine-GABA receptor chloride ionophore complex has been associated with the actions of many anxiolytic agents, there are several clinically effective anxiolytics whose mechanisms of action are obscure. The availability of new neuropharmacologic tools has permitted the examination of whether there are other neurochemical pathways involved in the actions of such "atypical" anxiolytics. Buspirone, a non-benzodiazepine anxiolytic has been

examined with the use of these agents. Buspirone elicits an anticonflict (anxiolytic) action in both rats and monkeys. Nonetheless, the anticonflict actions of this compound could not be blocked by either Ro 15-1788 or CGS-8216, suggesting that benzodiazepine receptors are not directly involved in mediating the pharmacologic actions of this compound. Nonetheless, pretreatment of animals with anxiolytic doses of buspirone, followed by injection of a "tracer" dose of [^3H]diazepam, results in an increased amount of [^3H]diazepam binding in vivo. These results suggest that buspirone's anxiolytic actions may involve an indirect effect on benzodiazepine receptors.

In addition to the presence of stereospecific receptors for benzodiazepine receptors in the CNS, binding sites for [^3H]diazepam have also been described in both peripheral tissues (e.g., kidney) and transformed cells of neural origin. The profile of these sites is distinctly different from those found in the CNS. The function of these sites is not known. However, recent studies have also demonstrated the presence of these sites in the CNS using [^3H]Ro 5-4864 (4'-chlorodiazepam), the prototype benzodiazepine active at this site ($K_i > 1 \text{ nM}$). This compound is inactive at central benzodiazepine receptors ($K_i > 100 \text{ }\mu\text{M}$). The lack of knowledge of the function of this site may be attributed to the apparent lack of pharmacologic action of the prototypic compound active at this site, Ro 5-4864. Recently, it was discovered that the guinea pig, in contrast to the rat, has a very high density of these sites in the CNS which suggests this species may provide a valuable model to determine the pharmacologic and physiologic role of these sites in both the periphery and the CNS. Parenteral administration of Ro 5-4864 elicited convulsions in guinea pig. The potency of this compound (five times more potent than the standard chemical convulsant pentylenetetrazol) suggests this was not a "nonspecific" action. The profile of compounds which antagonize these convulsions suggests that Ro 5-4864 does not act directly at "brain-type" benzodiazepine receptors. However, an action at [^3H]Ro 5-4864 high affinity binding sites in brain cannot be ruled out. The convulsions elicited by Ro 5-4864 are qualitatively different than those observed after other convulsants but closely resemble those observed after limbic (e.g., amygdaloid) kindling, insofar as there is a very prominent facial and forelimb clonus.

Purification of the benzodiazepine/GABA receptor complex using affinity chromatography and various immunological techniques is currently underway. Using an affinity gel coupled to the pharmacologically active benzodiazepine Ro 7-1986, it has been possible to purify the benzodiazepine receptor approximately 1000 fold. Current efforts are aimed at scaling up the purification procedure to a preparative scale in order to isolate enough receptor protein for the development of monoclonal antibodies.

Significance to Biomedical Research and the Program of the Institute:

The benzodiazepines and other minor tranquilizers described in these studies are widely prescribed and/or abused substances. Understanding the mechanisms by which these compound exert their pharmacologic actions are of fundamental importance to a better understanding of epilepsy, anxiety-neuroses, sleep disorders, and depression. These studies may also help clarify the role of ion transport and electrical conductance in the mechanisms of action of these agents and may be of fundamental significance in determining the role of ion transport in various pathological states. These studies can provide valuable information

leading to the development of more efficacious therapeutic agents which lack major side effects.

Proposed Course of Project:

Electrophysiological studies are planned to determine if annelated benzodiazepines can augment the electrophysiological actions of GABA in situ, since the in vitro results obtained may not mimic the in vivo situation. These findings will be important in determining the relationship of GABA to the pharmacologic actions of benzodiazepine-like compounds. Studies are planned to determine the effects of chronic treatment with "atypical" anxiolytics in both rats and primates to determine if the pharmacological actions of these compounds are altered. The ability of a number of pharmacologically active anticonvulsants will be examined for their abilities to modify Ro 5-4864 induced convulsions. This will aid in determining the mechanism of action of Ro 5-4864. Furthermore, electrophysiological and electroencephalographic studies Ro 5-4864 and other types of chemical convulsants that are associated with benzodiazepine receptors (e.g., pentylenetetrazole). The effects of Ro 5-4864 will be explored in the amygdaloid kindling model of epilepsy and genetically seizure prone (audiogenic) animals). Biochemical studies with [³H]Ro 5-4864 binding in various subcellular preparations of rat brain will be carried out in order to identify the possible sites of action. In addition, behavioral studies in both rodents and primates will be conducted to clarify any unique or common behavioral effects of Ro 5-4864 compared to other benzodiazepines.

Publications:

1. Cain, M., Weber, R., Guzman, F., Cook, J., Barker, S., Rice, K., Crawley, J., Paul, S.M. and Skolnick, P. β -Carbolines: Synthesis, neurochemical, and pharmacologic actions on brain benzodiazepine receptors. J. Med. Chem. 25: 1081-1091, 1982.
2. Ninan, P.T., Insel, T.R., Cohen, R.M., Cook, J.M., Skolnick, P. and Paul, S.M. Benzodiazepine receptor mediated experimental "anxiety" in primates. Science 218: 1332-1334, 1982.
3. Paul, S.M., Marangos, P., Skolnick, P. and Goodwin, F. Biological substrates of anxiety: benzodiazepine receptors and endogenous ligands. l'Encephale 8: 131-144, 1982.
4. Paul, S.M. and Skolnick, P. Comparative neuropharmacology of antianxiety drugs. Pharmacol. Biochem. Behav. 17, suppl 1: 37-41, 1982.
5. Schweri, M., Cain, M., Cook, J., Paul, S.M. and Skolnick, P. Blockade of 3-carbomethoxy- β -carboline induced seizures by diazepam and the benzodiazepine antagonists, Ro 15-1788 and CGS 8216. Pharmacol. Biochem. Behav. 17: 457-460, 1982.
6. Skolnick, P., Marangos, P. and Paul, S.M. Putative endogenous ligands of the benzodiazepine receptor. In Malick, J., Enna, S. and Yamamura, H. (Eds.): Anxiolytics. New York, Raven Press, 1982, pp. 41-53.

7. Skolnick, P. and Paul, S. Buspirone: chemistry, pharmacology, and behavior. J. Clin. Psychiatry 43: 40-42, 1982.
8. Skolnick, P., Paul, S. and Rice, K. Alkylating benzodiazepines: irazepine and kenazepine (monograph). Drugs of the Future 7: 257-259, 1982.
9. Skolnick, P., Schweri, M., Kutter, E., Williams, E. and Paul, S. Inhibition of [^3H]diazepam and 3-carboethoxy- β -carboline binding by irazepine: evidence for binding to different "domains" of the benzodiazepine receptor. J. Neurochem. 39: 1142-1146, 1982.
10. Skolnick, P., Williams, E.F., Cook, J.M., Cain, M., Rice, K.C., Mendelson, W.B., Crawley, J.N. and Paul, S.M. β -Carbolines and benzodiazepine receptors: structure-activity relationships and pharmacologic activity. In Usdin, E. (Ed.): β -Carbolines and Tetrahydroisoquinolines. New York, Alan R. Liss, Inc., 1982, pp. 233-252.
11. Paul, S., Luu, M.D. and Skolnick, P. The effects of benzodiazepines on presynaptic calcium transport. In Usdin, E., Skolnick, Tallman, J., Greenblatt, D. and Paul, S. (Eds.): Pharmacology of Benzodiazepines. London, Macmillan Press, 1983, pp. 87-92.
12. Skolnick, P., Hommer, D. and Paul, S.M. Benzodiazepine antagonists. In Usdin, W., Skolnick, P., Tallman, J., Greenblatt, D. and Paul, S. (Eds.): Pharmacology of Benzodiazepines. London, Macmillan Press, 1983, pp. 441-454.
13. Skolnick, P., Marangos, P.J. and Paul, S.M. The role of benzodiazepine receptors in seizures. In Delgado-Escueta, A.V. and Wasterlain, C. (Eds.): Proceedings of International Symposium on Status Epilepticus. New York, Raven Press, 1983, pp. 359-364.
14. Skolnick, P., Schweri, M., Paul, S., Martin, J. and Mendelson, W. 3-Carboethoxy- β -carboline (β -CCE) elicits electroencephalographic seizures in rats: reversal by the benzodiazepine antagonist, CGS 8216. Life Sci. 32: 2439-2445, 1983.
15. Skolnick, P. and Paul, S. Commentary: Purines and the regulation of food intake: an integrated approach. Integrative Psychiatry 1: 10-11, 1983.
16. Schweri, M., Paul, S. and Skolnick, P. Strain differences in convulsive susceptibility to 3-carbomethoxy- β -carboline. Pharmacol. Biochem. Behav., in press.
17. Schweri, M., Martin, J., Mendelson, W., Barrett, J., Paul, S. and Skolnick, P. Pharmacokinetic and pharmacodynamic factors contributing to the convulsant actions of β -carboline-3-carboxylate esters. Life Sci., in press.
18. Skolnick, P. and Paul, S. New concepts in the neurobiology of anxiety. J. Clin. Psychiatry, in press.
19. Weissman, B.A., Cott, J., Paul, S.M. and Skolnick, P. Ro 5-4864: A potent benzodiazepine convulsant. Eur. J. Pharmacol., in press.

20. Paul, S.M. and Skolnick, P. The Biochemistry of Anxiety: From Pharmacology to Pathophysiology in Psychiatry Update, vol. III. The American Psychiatric Association Annual Review, APA Press, Washington, D.C., in press.
21. Paul, S.M., Ninan, P., Insel, T. and Skolnick, P. Benzodiazepine receptor-mediated experimental "anxiety" in rhesus monkeys. In Gram, L. and Udsin, E. (Eds.): Proceedings of the Third International Meeting on Clinical Pharmacology in Psychiatry. London, Macmillan Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02186-01 NS

PERIOD COVERED
October 1, 1982 through September 30, 1983

Brain Recognition

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Sites for Stimulants and Antidepressants: Relationship to Pharmacological Activity

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Steven M. Paul, Chief, Clinical Neuroscience Branch, NIMH

COOPERATING UNITS (if any)

Laboratory of Bioorganic Chemistry, NIADDK; Georgetown University, Washington, D.C; Section on Molecular Pharmacology, NS, NIMH.

Clinical Neuroscience Branch

LAB/BRANCH

Section on Preclinical Studies

SECTION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

INSTITUTE AND LOCATION

4.9
TOTAL MANYEARS:

4.3
PROFESSIONAL:

0.6
OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☒ (b) Human tissues

☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Recognition sites for a variety of psychotherapeutic drugs have been identified in the mammalian central nervous system. Several of these binding sites, including those for benzodiazepines, opiates, and various neuroleptics have subsequently been shown to be true pharmacological receptors in that the binding of drug to its respective recognition site is a necessary (and many times sufficient) requirement for drug action. Over the past several years we have attempted to identify recognition sites for other common psychotropic drugs including tricyclic antidepressants and the psychomotor stimulants, amphetamine and methylphenidate. In each case relatively high affinity, saturable, and stereospecific binding sites have been delineated; and for amphetamine and methylphenidate relatively good correlations have been observed between the affinities of a series of analogues in vitro and at least some of the pharmacological properties of these agents. Tricyclic antidepressants including imipramine and desipramine, also bind to distinct recognition sites that are functionally and structurally associated with the presynaptic uptake sites for serotonin and norepinephrine respectively. Thus, radiolabelled antidepressants have been useful probes in studying the mechanisms of neurotransmitter uptake in both central and peripheral tissues, and under a variety of clinical conditions. Studies on elucidating the possible physiological significance of these binding sites as well as their relationship to pathophysiological mechanisms of neuropsychiatric disease are in progress.

Other Professional Personnel:

Phil Skolnick	Pharmacologist	LBC NIADDK
Itzhak Angel	Guest Worker	NS NIMH
Aaron J. Janowsky	Guest Worker	PRAT NIGMS
Richard L. Hauger	Staff Fellow	NS NIMH
Rodrigo Labarca	Guest Worker	NS NIMH
Jacqueline N. Crawley	Res. Biologist	NS NIMH
Rochelle Schwartz	Pharmacologist	NS NIMH
Margaret M. Schweri	Staff Fellow	LPB NIADDK
Michael Rafferty	Staff Fellow	LC NIADDK
Kenner Rice	Staff Fellow	LC NIADDK
Moshe Rehavi	Guest Worker	NS NIMH
Emmy Truckenmiller	Graduate Student	Georgetown Univ.
Joseph Neale	Professor	Georgetown Univ.

Project Description:Methods Employed:

Radioreceptor techniques using radioactive ligands of high specific radioactivity have been employed for the neurochemical identification and characterization of specific drug recognition sites. Light microscopic autoradiography has also been employed for the subsequent analysis of the regional distribution of these binding sites in brain. Behavioral studies in rodents are conducted to compare the pharmacological (viz. behavioral) potencies of drugs with their in vitro potencies at their respective binding sites. In vitro neurotransmitter uptake studies conducted in crude and partially purified synaptosomal fractions are performed as a functional measure in studies where drugs that potentially block reuptake (e.g. antidepressants, methylphenidate and amphetamines) are being studied.

Major Findings:

High affinity, stereospecific binding sites for [^3H](+)-amphetamine have been described in rodent brain. The highest density of these sites are found in synaptosomal fractions of brain stem and hypothalamus. A striking correlation ($r = 0.97$; $p < .01$) has been demonstrated between the ability of a series of amphetamine derivatives to displace [^3H](+)-amphetamine from these sites and their potencies as anorexic agents. A similar correlation was not observed between in vitro potency and motor stimulation. These observations suggested that this site may be involved in the appetite suppressant actions of amphetamine. [^3H](+)-Amphetamine binding has also been studied in a genetically obese mouse strain, ob/ob. In these animals, the density of [^3H](+)-amphetamine binding sites is approximately 30-50% higher than in litter mate controls. Furthermore, food deprivation of rats (72 hours) results in a dramatic (30-50%) reduction in the density of hypothalamic [^3H](+)-amphetamine binding sites. Refeeding the animals for a four hour period results in a return to the control densities of these binding sites. These data suggest that the [^3H](+)-amphetamine binding sites may be intimately involved in the feeding behavior of animals.

High affinity, stereospecific binding sites have also been described for another psychomotor stimulant [^3H] methylphenidate. These sites are highly localized to the synaptosomal fraction of striatum. Low densities are found in the hypothalamus and brain stem. A high correlation ($r = 0.81$; $p < 0.05$) has been found between the ability of a series of methylphenidate derivatives to stimulate motor activity and their abilities to compete with [^3H] methylphenidate for these binding sites. Furthermore, other psychomotor stimulants such as amphetamine and cathinone stereospecifically displace [^3H] methylphenidate binding in the striatum suggesting that a variety of psychomotor stimulants may bind to these sites; and that such binding may be pharmacologically relevant. Recent studies using specific neurotoxins such as 6 hydroxydopamine and 5,7 dihydroxytryptamine have shown that the majority of [^3H] methylphenidate binding sites are localized to presynaptic dopamine-containing neuronal elements.

The binding of [^3H] phencyclidine (PCP) has been shown to be altered in the presence of the calcium channel blocker, nifedipine. Furthermore, the increases in motor activity elicited by phencyclidine are reduced in a dose-dependent fashion by nifedipine. This action is not due to the hypotensive actions of nifedipine since hypotensive doses of the α -adrenoceptor blocker, prazosine, do not antagonize the motor stimulant properties of phencyclidine. These observations suggest that alterations of calcium channel permeability in the C.N.S. may provide a means of pharmacologically antagonizing some of the actions of phencyclidine.

Destruction of presynaptic catecholaminergic terminals by the neurotoxin 6-hydroxydopamine also resulted in a significant loss in binding sites for [^3H] phencycline. These observations suggest that "phencyclidine receptors" may be associated with presynaptic catecholamine terminals in the C.N.S., consonant with many of the reported electrophysiological actions of this compound.

Previous studies in our laboratory have demonstrated that [^3H] imipramine labels the "serotonin transporter" (recognition site + transport protein) in brain and platelets of both human and rat. The number of these sites is reduced in platelets of depressed patients, suggesting that this parameter could be an important marker for depressive illness. The demonstration of a strong concordance of [^3H] imipramine binding to platelets from monozygotic (identical) twins further supports the contention that this parameter may be a useful "biological marker" in depression. In followup of our initial work on [^3H] imipramine binding we have successfully solubilized the [^3H] imipramine binding site from human platelets with the ultimate goal of reconstitution of the serotonin transport system in artificial membranes. More recently we have immunized BALB/C mice with a partially purified human platelet membrane preparation and have generated monoclonal antibodies directed against platelet membranes. After screening over 900 hybrid cell colonies we have observed more than 100 antibodies that reacted with platelet membranes. Of these, several markedly influenced the uptake of serotonin and (or) [^3H] imipramine binding in platelets. Further a number of these same antibodies cross-reacted with rat brain synaptosomal membranes and altered serotonin uptake and [^3H] imipramine binding in this tissue as well. Studies are now in progress to completely characterize the various monoclonal antibodies and to identify whether any are specifically directed against the serotonin transporter and (or) [^3H] imipramine binding site.

Proposed Course of the Project:

Studies will be continued on the various recognition sites identified over the past 12 months in order to more fully elucidate their pharmacological as well as physiological significance. A variety of techniques including SDS/PAGE electrophoresis immunoprecipitation and various chromatographic methods will be applied to the purification and characterization of several of these binding sites and particularly the [^3H] imipramine and [^3H] desipramine recognition sites. The production of specific monoclonal antibodies directed against many of these protein(s) will be carried out to aid in their complete characterization.

Significance to Biomedical Research and the Program of the Institute:

All of the drugs under investigation have important psychotropic actions and are either of therapeutic benefit or reliably mimic various behavioral states. Thus an understanding of their mechanisms of action will be of undoubted value to understanding the behavioral and psychopathological states responsive to treatment with these agents.

Publications:

Paul, S.M., Hulihan, B., and Skolnick, P.: High affinity and stereospecific binding of [^3H]d-amphetamine to rat brain. Eur. J. Pharmacol. 78: 145-147, 1982.

Hauger, R.L., Skolnick, P., and Paul, S.M.: Specific [^3H] β -phenethylamine binding sites in rat brain. Eur. J. Pharmacol. 83: 147-148, 1982.

Paul, S.M., Hulihan-Giblin, B., and Skolnick, P.: (+)-Amphetamine binding in rat hypothalamus: Relation to anorexic potency of phenylethylamines. Science 218: 1332-1334, 1982.

Ittah, Y., Rehavi, M., Skolnick, P., Rice, K., and Paul, S.M.: Nitroimipramines: Selective, irreversible inhibitors of [^3H]serotonin uptake and [^3H]imipramine binding in platelets. Naunyn-Schmiedeberg's Arch. Pharmacol. 320: 45-49, 1982.

Hays, S.E. and Paul, S.M.: CCK receptors and human neurological disease. Life Sci. 31: 319-322, 1982.

Rehavi, M., Skolnick, P., and Paul, S.M.: Characterization of high affinity antidepressant binding to rat and human brain. In Gram, L., and Usdin, E. (Eds.): Proceedings of the Third International Meeting on Clinical Pharmacology in Psychiatry. London, MacMillan Press, in press, 1982.

Rehavi, M., Skolnick, P., and Paul, S.M.: Subcellular distribution of high affinity [^3H] imipramine binding and [^3H] serotonin uptake in rat brain. Eur. J. Pharmacol. 87: 335-339, 1983.

Paul, S.M., Rehavi, M., Skolnick, P., and Goodwin, F.K.: [^3H]Imipramine binding to the serotonin "transporter" in human brain and platelet: A possible biological marker in depression. In Hanin, I., and Usdin, E. (Eds.): Biological Markers in Psychiatry and Neurology. New York, Pergamon Press, pp. 193-204, 1982.

Paul, S.M., Rehavi, M., Nurnberger, J., Gershon, E., Skolnick, P., and Goodwin, F.K.: Concordance of [^3H] imipramine binding but not [^3H] serotonin uptake in latelets from monozygotic twins. Psychiatry Res., in press, 1983.

Hauger, R.L. and Paul, S.M.: Neurotransmitter receptor plasticity: Alterations by antidepressants and antipsychotics. Psychiatric Annals 13:5 399-407, 1983.

Rehavi, M., Skolnick, P., and Paul, S.M.: Solubilization and partial purification of high affinity [^3H] imipramine binding sites in rat brain. FEBS Lett. 150: 514-518, 1983.

Paul, S.M., Rehavi, M., Skolnick, P., and Goodwin, F.K.: [^3H]Imipramine binding to serotonin uptake sites in human brain and platelet: Studies in depression. In Post, R.M., and Ballenger, J.C. (Eds.): The Neurobiology of Mood Disorders. Baltimore, Williams and Wilkins, in press, 1983.

Rehavi, M., Tracer, H., Rice, K., Skolnick, P., and Paul, S.M.: [^3H]Nitroimipramine: A selective, "slowly-dissociating" probe of the imipramine binding site ("serotonin transporter") in platelets and brain. Life Sci. 32: 645-653, 1983.

Paul, S.M., Purdy, R.H., Hoffman, A.R., and Axelrod, J.: Radioenzymatic assays for catechol estrogens. In Merriam, G.R., and Lipsett, M.B. (Eds.): Catechol Estrogens. Raven Press, New York, pp. 83-90, 1983.

Hauger, R.L., Skolnick, P., and Paul, S.M.: Brain recognition sites for typical and atypical antidepressants. In Burrows, G.D. and Werry, J.S. (Eds.): Advances in Human Psychopharmacology, Vol. IV, in press, 1983.

Paul, S.M., Hauger, R.L., and Skolnick, P.: The effects of antidepressants on neurotransmitter receptors: Implications for their mechanism(s) of action. In Ayd, F.J., Taylor, I.J., and Taylor, B.T. (Eds.): Affective Disorders Reassessed, 1983, Ayd Medical Communication, Baltimore, Maryland, in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00112-06 NS

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Endorphin Research in Mental Illness

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

David Pickar, M.D., Chief, Section on Clinical Studies, NSB, NIMH

COOPERATING UNITS (if any)

Clinical Neuropharmacology Branch, NIMH; Surgery Branch, NCI; Department of Anesthesia, Georgetown Medical School; Fairfax Hospital

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Clinical Studies

INSTITUTE AND LOCATION

NIMH, ADAMHA, Bethesda, MD 20205

TOTAL MANYEARS:

9

PROFESSIONAL:

7

OTHER:

2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

This project studies the role of the endogenous opioid system (EOS) in humans. We have completed a dose-response study in normals of high doses of the opiate antagonist, naloxone (up to 4 mg/kg). Significant dose-dependent increases in physiologic (BP and respiratory rate) and hormonal variables (cortisol and growth hormone) were found suggesting progressive EOS blockade with increasing naloxone doses. Normals also experienced dysphoria at high naloxone doses suggesting EOS involvement in the regulation of mood in normals. In a separate double-blind study, high doses of naloxone produced a significant decrease in caloric intake in healthy, normal volunteers supporting hypothesized involvement of the EOS in eating behavior. In other work, we have studied the relationship between the hypothalamic-pituitary-adrenal axis and the EOS in depressive illness. As a continuation of our work demonstrating the responsiveness of the EOS to stress and involvement of the EOS in endogenous analgesic mechanisms, we have initiated a study of the analgesic and behavioral effects of synthetic β -endorphin administered by lumbar intrathecal route to patients with metastatic disease. In the two patients studied to date, intrathecally administered β -endorphin produced long-lasting analgesia. In addition, one of the patients experienced a unique behavioral syndrome characterized by hypomania/mania, psychosis and confusion. Further work is needed with this strategy to more fully evaluate analgesic potentials and the behavioral effects of this endogenous peptide when administered with access to the CNS.

OTHER PROFESSIONAL PERSONNEL

Martin R. Cohen, M.D.	Staff Psychiatrist	NSB	NIMH
Robert M. Cohen, M.D./Ph.D.	Chief, Clinical Brain Imaging Section	LPP	NIMH
Dennis L. Murphy, M.D.	Chief	CNB	NIMH
Thomas Insel, M.D.	Staff Psychiatrist	CNB	NIMH
Paul Sugarbaker, M.D.	Senior Investigator, Surgery Branch		NCI
Michel Dubois, M.D.	Staff Member, Dept. of Anesthesia, Georgetown Medical School		
Thomas N. Wise, M.D.	Chief of Psychiatry, Fairfax Hospital		

PROJECT DESCRIPTION

This is a continuation of work begun in the Biological Psychiatry Branch and continuing in the Clinical Neuroscience Branch. The major aim of this project is to study the roles of the endogenous opioid system (EOS) in human behavior and physiology and in psychiatric illness. During the past year we have continued and refined our use of the naloxone administration strategy to study the tonic role of the EOS in humans. We have further studied links between hypothalamic-pituitary-adrenal (HPA) axis and the EOS in depressed patients. Finally, we have continued to investigate the response of endogenous opioids to stress and its relationship with endogenous analgesic mechanisms.

Methodology:

I. The Administration of High-Dose Naloxone

Dr. Martin R. Cohen has coordinated this work intending to study tonic aspects of the endogenous opioid system in humans. Towards this end, we have developed the methodology of administering larger doses of the opiate antagonist, naloxone, than had been previously administered in the clinical setting. Doses up to 4 mg/kg have been administered in a dose-response study to normal volunteers. Dose-response changes in behavior, physiologic function and neuroendocrine variables have been examined to gain a better understanding of the optimal dose of naloxone to ensure EOS blockade and the tonic role of endorphins in humans.

II. Plasma Measurements

We have continued to use the strategy of measuring plasma levels of β -endorphin immunoreactivity (ir) in the clinical setting, particularly to study relationships between levels of plasma β -endorphin (ir) and levels of plasma cortisol in depression.

III. Intrathecal β -Endorphin Administration

We have previously demonstrated that the EOS, as reflected by levels of plasma β -endorphin (ir), is extremely responsive to the severe physical stress of abdominal surgery. We, furthermore, demonstrated that levels of β -endorphin (ir) stimulated by surgical stress predicted (inversely) the amount of post-operative analgesic required to control the patient's pain. We have continued our interest in endogenous analgesic and stress aspects

of the EOS. Towards this end, we have developed, in collaboration with the National Cancer Institute, a protocol in which 3 mg of synthetic β -endorphin is administered intrathecally (lumbar route) to patients with severe pain secondary to malignancy. This project intends to study the reported high-potency analgesic effects of β -endorphin when so administered as well as the behavioral effects of this endogenous peptide when administered in pharmacologic doses with access to the CNS. This project requires intensive medical monitoring and is carried out in a surgical intensive care unit of the NIH Clinical Center.

MAJOR FINDINGS

1. Previous clinical studies using the opiate receptor antagonist, naloxone, have shown little or inconsistent behavioral effects in normal humans. In order to assess the notion that previous doses used were insufficient to yield a complete EOS blockade, normal volunteers were administered increasing doses of naloxone (0.3-4.0 mg/kg) in a single-blind study. We have observed significant dose-dependent behavioral, hormonal (cortisol and growth hormone) and physiological effects associated with increasing doses of naloxone. With high naloxone doses, volunteers experienced increasing dysphoria, a deterioration of performance on memory testing, increased systolic blood pressure and respiratory rate. These results suggest that lower doses of naloxone used in previous clinical studies may not have been sufficient to produce complete EOS blockade, and indicate involvement of EOS in the tonic regulation of normal human mood, memory, BP, respirations, plasma growth hormone and cortisol levels. In a separate double-blind study using a 2 mg/kg dose of naloxone administered to 7 normal volunteers, naloxone significantly reduced total food intake from preselected prepared trays served at 2.75 and 7.75 hours following drug administration. These data are consistent with animal studies demonstrating EOS modulation of food intake and suggest further studies for use of naloxone in treating eating disorders.

2. We have measured plasma β -endorphin in groups of patients with major depressive and minor depressive disorder and normal controls. Minor depressives had significantly less plasma β -endorphin than did patients with major depression or controls. In contrast, patients with major depressive disorder demonstrated predictable elevations in plasma cortisol compared to the other study groups and in major depressives, but not other groups, plasma β -endorphin was directly correlated with plasma cortisol. This finding complements earlier work from our group suggesting a link between the HPA axis and the EOS in major depression.

We have also studied the relationship between the EOS and HPA axis in obesity in collaboration with Fairfax Hospital. Morning plasma cortisol and β -endorphin levels were found to be no different in obese patients, prior to diet treatment, than normal weight relatives. Plasma cortisol levels were significantly correlated in obese patients with self-ratings of depression. During the course of a 400 calorie per day modified protein fast we observed significant decreases in levels of plasma cortisol but unchanging levels of plasma β -endorphin. Patients who failed to complete the 6-month diet program, however, were found to have had significantly higher levels of

plasma β -endorphin on entry into the program than those who were able to complete the diet program.

3. We have administered 3 mg of β -endorphin, by lumbar intrathecal route, to 2 patients with disseminated malignancy. We observed that β -endorphin produced profound and long-lasting analgesia. The first patient experienced analgesia lasting over 16 hours; the second patient experienced analgesia lasting over 60 hours. Though there was a suggestion of enhanced mood in the first subject, the second subject experienced a significant behavioral effect. This behavioral syndrome was characterized by confusion, hypomanic/manic behavior and psychosis and lasted for over 2 days. We are continuing to evaluate this methodology for future studies in which both the analgesic and behavioral effects of β -endorphin are to be studied.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

The endogenous opioid system represents one of the most important scientific discoveries of the last decade. Our program, aiming to discern the roles of this system in humans, is of considerable importance not only to psychiatry but to the overall field of medicine. Our work, using high doses of naloxone has added to methodologic approaches in studying the endogenous opioid system and has provided new evidence suggesting tonic roles for this system in human behavior, physiology and neuroendocrine regulation. We, furthermore, have evidence suggesting a role for the endogenous opioid system in eating behavior in normal subjects.

Our work in psychiatric patients has continued to suggest important relationships between the HPA axis and the EOS in depression. This work is of particular significance since abnormalities in HPA axis function have been a consistent and to date unexplained finding in depression.

The strategy of intrathecal β -endorphin administration is important for several reasons. First, it represents an aggressive treatment/research technique in which substances (e.g., peptides) which do not readily cross the blood-brain barrier following peripheral administration can now be studied with regard to CNS effects. Furthermore, it appears that β -endorphin itself may be an extremely potent analgesic which may alleviate suffering in patients with chronic and severe pain. Furthermore, our data support the notion that β -endorphin itself may have important behavioral properties in humans.

PROPOSED COURSE

We intend to continue our studies using naloxone both in normal and abnormal states. Furthermore, we intend to utilize naloxone strategies in depressed, schizophrenic and normal subjects with particular attention to differential HPA axis responses between groups. Finally, the difficult but potentially important strategy of intrathecal drug administration will be pursued with the National Cancer Institute.

PUBLICATIONS

- Naber, D., Pickar, D.: Endorphins in CSF and plasma of psychiatric patients. Drug Res. 32: 877-878, 1982.
- Cohen, M.R., Cohen, R.M., Pickar, D., Murphy, D.L., Bunney, W.E., Jr.: Physiological effects of high dose naloxone administration to normal adults. Life Sciences 30: 2025-2031, 1982.
- Pickar, D., Extein, I., Gold, P.W., Naber, D., Summers, R.S., Goodwin, F.K.: Endorphins and affective disorders. In Shah, N.S. and Donald, A.G. (eds.). Endorphins and Opiate Antagonists in Psychiatric Research. New York, Plenum Press, pp 375-398, 1982.
- Pickar, D., Naber, D., Post, R.M., van Kammen, D.P., Kaye, W., Rubinow, D., Ballenger, J.C., Bunney, W.E. Jr.: Endorphins in CSF of psychiatric patients. In Opioids in Mental Illness. Annals of New York Academy of Sciences 398:399-412, 1982.
- Cohen, R.M., Pickar, D., Dubois, M., Bunney, W.E., Jr: Clinical studies of stress and the endogenous opioid system. In Opioids in Mental Illness. Annals of New York Academy of Sciences 398:424-432, 1982.
- Pickar, D., Cohen, M.R., Naber, D., Cohen, R.M.: Clinical studies of the endogenous opioid system. Biological Psychiatry 17:1243-1276, 1982.
- Dubois, M., Pickar, D., Cohen, M.R., Gadde, P., Macnamara, T.E., Bunney, W.E., Jr: Effects of fentanyl on the response of plasma beta-endorphin to surgery. Anesthesiology 57:468-472, 1982.
- Schulz, S.C., van Kammen, D.P., Pickar, D., Cohen, M.R., Naber, D.: Response of plasma beta-endorphin immunoreactivity to d-amphetamine and placebo in schizophrenic patients. Psychiatry Res. 7:177-178, 1982
- Buchsbaum, M.S., Davis, G.C., Naber, D., Pickar, D.: Pain enhances naloxone hyperalgesia in man. Psychopharmacology, 79:99-103, 1983.
- Pickar, D., Cohen, M.R., Dubois, M.: The relationship of plasma cortisol and β -endorphin immunoreactivity to surgical stress and post-operative analgesic requirement. General Hospital Psychiatry, 5:93-98, 1983.
- Pickar, D., Cohen, M.R., Naber, D., Post, R.M.: The endogenous opioid system in human behavior. In Pancheri, P., Zichella, L. (eds.). Psycho-Neuro-Endocrinology in Reproduction. New York, Raven Press, in press.
- Cohen, M.R., Cohen, R.M., Pickar, D., Murphy, D.L., Bunney, W.E., Jr. High-dose naloxone administration produces dose-dependent behavior and hormonal effects in normal humans. Arch. Gen. Psychiatry, 40:613-619, 1983.

- Kaye, W.H., Pickar, D., Ebert, M.H., Naber, D. The opioid system in anorexia nervosa. Am. J. Psychiatry 140:371-372, 1983.
- Pickar, D. Naloxone in schizophrenia. Lancet i:819, 1983.
- Cohen, R.M., Pickar, D., Extein, I., Gold, M.S., Sweeney, D.R. Plasma cortisol and β -endorphin immunoreactivity in nonmajor and major depression. Am. J. Psychiatry, in press.
- Naber, D., Bullinger, M. and Pickar, D. Neuroendocrine, psychological and psychophysiological variables in human stress response. In Pancheri, P., Zichelli, L. (eds) Psycho-neuro-endocrinology of Human Reproduction, New York, Raven Press, in press.
- Naber, D., Pickar, D. The measurement of endorphins in body fluids. In Risch, S.C., Pickar, D. (eds) Psychiatric Clinics of North America: Advances in Endorphin Research, Philadelphia, W. B. Saunders Co., in press.
- Cohen, M.R., Pickar, D., Dubois, M. The role of the endogenous opioid system in the human stress response. In Risch, S.C., Pickar, D. (eds). Psychiatric Clinics of North America: Advances in Endorphin Research, Philadelphia, W. B. Saunders Co., in press.
- Cohen, M.R., Pickar, D., Cohen, R.M., Wise, T.N., Copper, J.N. Plasma cortisol and beta-endorphin immunoreactivity in human obesity. Psychosomatic Medicine, in press.
- Insel, T.R., Pickar, D. Naloxone administration in obsessive-compulsive disorder: Report of two cases. Am. J. Psychiatry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02181-01 NS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurobiology of Schizophrenia		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David Pickar, M.D., Chief, Section on Clinical Studies, NS, NIMH		
COOPERATING UNITS (if any) Office of Clinical Director, NIAAA; Laboratory of Psychology and Psychopathology, NIMH; Adult Psychiatry Branch, St. Elizabeth's Hospital, NIMH; Emory University, Atlanta, GA; Sheppard Pratt Hospital, Baltimore, MD		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, MD 20205		
TOTAL MANYEARS: <div style="text-align: center;">14</div>	PROFESSIONAL: <div style="text-align: center;">11</div>	OTHER: <div style="text-align: center;">3</div>
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The aim of this project is to gain greater understanding into the <u>psychobiology</u> of the illness of <u>schizophrenia</u>. Three principal areas of research have been begun during the past year: the <u>phenomenology</u> and bio-chemistry of <u>neuroleptic response</u>, <u>neuropsychiatric aspects</u> of schizophrenia, and clinical and biological <u>correlates</u> of depressive and "negative" symptoms in schizophrenia. </p> <p> Clinical research for this project is carried out on the 4-East Nursing Service of the Clinical Center. Patients with DSM III diagnosed schizophrenia are studied during a several month research period during which they are administered neuroleptic and placebo medications under double-blind conditions. Plasma determinations for levels of HVA, MHPG and 5-HIAA are performed throughout this period of study. Clinical changes associated with neuroleptic administration and discontinuation are examined with relationship to levels of amine metabolites. This portion of the project is aimed towards gaining a better understanding of the mechanism of neuroleptic action in schizophrenia. </p> <p> Recent high-technology strategies have become available in clinical neuroscience research. <u>Computer axis tomography</u>, <u>positron emission tomography</u> and sophisticated evoked potential systems are now available for use in the study of schizophrenia. We have attempted to utilize these strategies to study possible neuroanatomic and neurophysiologic abnormalities which may relate to the etiology or pathophysiology of schizophrenia. </p> <p> A third portion of this research program studies depressive and "negative" symptomatology during the course of the schizophrenia illness. This work includes the application to patients with schizophrenia of biological tests currently employed in studying depressive illness and phenomenologic study of interrelationships between depressive and "negative" symptoms. Systematic investigation into <u>suicidal behavior</u> in schizophrenic patients is also currently in progress. </p> <div style="text-align: right;">(721)</div>		

OTHER PROFESSIONAL PERSONNEL

Steven M. Paul, M.D.	Chief	NSB	NIMH
Alec Roy, M.B.	Visiting Associate	NSB	NIMH
Owen Wolkowitz, M.D.	Clinical Associate	NSB	NIMH
Allen Doran, M.D.	Clinical Associate	NSB	NIMH
Judy Schreiber, M.S.W.	Social Worker	NSB	NIMH
Markku Linnoila, M.D.	Clinical Director		NIAAA
Alan Mirsky, Ph.D.	Chief	LPP	NIMH
Robert M. Cohen, M.D./Ph.D.	Chief, Clinical Brain Imaging Section	LPP	NIMH
Daniel Weinberger, M.D.	Chief, Clinical Neuropsychiatry and Neurobehavior Section	SMRA	NIMH
Ronald Zec, Ph.D.	Staff Fellow, Clinical Neuropsychiatry and Neurobehavior Section	SMRA	NIMH
John Boronow, M.D.	Psychiatrist, Sheppard Pratt Hospital Baltimore, MD		
Philip Ninan, M.D.	Psychiatrist, Emory University Clinic Atlanta, GA		

PROJECT DESCRIPTION

This project is part of the research program of the Section on Clinical Studies of the Clinical Neuroscience Branch. This section conducts clinical research based on the 4-East Nursing Unit of the Clinical Center.

The aim of this project is to gain greater understanding into the psychobiology of schizophrenia. Towards this end systematic research has been initiated in the following areas pertaining to schizophrenia: (1) the biochemistry and phenomenology of neuroleptic response; (2) etiologic perspectives from neuropsychiatry and (3) the biology of depressive and "negative" symptomatology in schizophrenia.

I. BIOCHEMISTRY AND PHENOMENOLOGY OF NEUROLEPTIC RESPONSE

Despite enormous research attempting to alleviate symptoms of schizophrenia, the group of drugs known as neuroleptics have remained over the last two decades as the principle pharmacologic agents in the treatment of schizophrenia. The close relationship between the affinities of representative neuroleptic drugs to bind to non-adenylcyclase dependent postsynaptic dopamine receptors and their clinical antipsychotic potencies represents the foundation for the dopamine hypothesis of schizophrenia. Despite the attractiveness of the notion of hyperactivity of dopamine systems in schizophrenia, considerable clinical data are inconsistent in its support. This portion of our research program attempts to systematically study the mechanism of neuroleptic action with particular attention to changes in dopamine system activity. Our goal is to attempt to link behavioral change associated with neuroleptic administration and discontinuation with multiple longitudinal determinants of plasma levels of monoamine metabolites. It is hoped that a greater understanding of the clinical response and non-response to neuroleptics in schizophrenia will provide leverage for evaluating and developing newer treatments for this severely debilitating illness.

Methodology:

A. Behavioral Assessment:

Patients are rated daily by the 4-East nursing staff using the Bunney-Hamburg Scale. Patients are maintained on double-blind medication throughout the research period which includes at least 4 weeks free from all medications. In addition to nurses' ratings, physicians, blind to patients' medication status, perform Bunney-Hamburg global ratings of psychosis and depression, the Brief Psychiatric Rating Scale, and the newly developed Taylor-Abrams Scale for the assessment of negative symptoms.

B. Plasma and CSF Monoamine Metabolites:

During the course of neuroleptic and placebo administrations, plasma is collected three times weekly for assessment of levels of monoamine metabolites. HVA, a major metabolite of dopamine, is examined with regard to the course of behavioral change associated with neuroleptic treatment. Plasma MHPG and 5HIAA is also examined during selected periods with particular emphasis in studying relationships with affective symptoms.

In addition to these plasma measurements, at least two lumbar punctures (while the patient is on and off neuroleptics) are performed during the patient's research period. CSF amine metabolites, norepinephrine and dopamine levels are determined and examined for possible relationships to behavioral change.

C. Additional Drug Treatment:

One important aspect of this study is the development of improved treatment strategies for patients with schizophrenia. Drugs which are known to affect CNS dopamine systems will be examined as additive therapeutic agents to neuroleptics. Such drugs may include dopamine agonists such as amantadine or catecholamine depleters such as reserpine.

II. ETIOLOGIC PERSPECTIVES FROM NEUROPSYCHIATRY

For many years researchers have attempted to delineate neuropathologic changes associated with the illness of schizophrenia. The advent of new technologies applicable to the clinical setting has created a resurgence in research attempting to identify specific neuroanatomic abnormalities in this illness. As part of our program in studying schizophrenia, we have attempted to utilize new and emerging technologies in conjunction with ongoing behavioral, biochemical and pharmacologic studies to develop a larger perspective of the pathophysiology of schizophrenia.

Methodology:

A. CAT Scan Studies:

Studies from centers throughout the world have now reported the occurrence of abnormalities in ventricular size in patients with schizophrenia. We have

utilized CAT scan technology to study size of lateral and third ventricles using planimetric methods and have determined sulcal atrophy in a sizable group of patients with schizophrenia and in a matched group of medical controls from the Clinical Center. We have also developed a protocol to study CAT scan determinations in non-medical controls.

B. PET Scan:

Positron emission tomography, one of the newest technologies applicable to studying brain function, is increasingly utilized as part of our research program. In collaboration with the Laboratory of Psychology and Psychopathology, we are developing better methods applicable to studying patients with schizophrenia during PET scan procedures. The goal here is to study possible abnormalities in the brain utilization of glucose during the performance of specific psychological tests. Collaborative effort has been given to develop selective tasks most applicable to patients with schizophrenia.

C. Neuropsychological Testing:

In collaboration with the Adult Psychiatry Branch at St. Elizabeths Hospital and with the Laboratory of Psychology and Psychopathology, we have developed a battery of neuropsychological testing for patients with schizophrenia. This work is intended to document and describe significant neuropsychological dysfunction in patients with schizophrenia. These data are intended to be examined with regard to drug response, biochemical measures and performance on neurophysiologic tests.

III. DEPRESSIVE AND "NEGATIVE" SYMPTOMATOLOGY IN SCHIZOPHRENIA

It has been appreciated for many years that patients with schizophrenia suffer from depressive symptoms as well as "negative" symptoms such as withdrawal, poverty of speech, etc. This portion of our research program studies the interrelationship between depressive and "negative" symptoms in schizophrenia and their relationship with biological tests shown to be useful in studying depressive illness.

Methodology:

A. Biological Tests for Depression:

Several biological tests have been reported to be useful in diagnosing and assessing patients with depressive illness. These include the dexamethasone suppression test, TSH response to TRH infusion, and platelet serotonin-uptake and imipramine-binding assays. We are applying these methods to patients with schizophrenia at two phases: when the patient is medication-free and again when the patient is treated with neuroleptic medication. These data are examined with regard to the emergence of depressive and "negative" symptoms in patients with schizophrenia and will be compared with results from our studies of patients with depressive illness.

B. Studies of Suicide:

Recent data have documented that the incidence of suicide in patients with schizophrenia is very similar to that of depression. We have begun systematic retrospective and prospective studies of suicidal behaviors in schizophrenia. These clinical data will be examined with regard to possible relationships to biochemical and other clinical indices accrued during patients stay on the research service.

MAJOR FINDINGS

The studies described above have been initiated during this past year and are currently in progress. Some preliminary data are, however, available.

With regard to neuroleptic treatment, our data suggest that with chronic treatment neuroleptic drugs may modulate or dampen CNS dopamine systems rather than merely "block" dopamine transmission. We are currently examining the possibility that decreased variation in dopamine activity, as reflected by levels of plasma HVA, may be more important to eventual neuroleptic response than simply a decrease in levels. These data may have important implications for further strategies involving dopamine systems in schizophrenia.

With regard to neuropsychiatric aspects of schizophrenia, a preliminary study in our patients was unable to identify abnormality in size of lateral ventricles in patients with schizophrenia. We were, however, able to demonstrate increased size of third ventricles in schizophrenia compared to control subjects, although within schizophrenic patients ventricular size did not relate to clinical or biochemical variables.

Preliminary data have demonstrated to us that lack of suppression to dexamethasone is not a finding unique to pure depressive illness. We have observed this phenomenon in a number of medication-free schizophrenics. We are currently completing a larger sample size and will be attempting to relate this phenomenon to the occurrence of depressive and "negative" symptoms.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

The illness of schizophrenia is a major public health problem in the United States. This project attempts to study etiologic factors in schizophrenia and to develop a better understanding of mechanisms underlying current pharmacologic treatments. New treatment strategies which may develop from this work would have considerable importance to the field of psychiatry and to the estimated 2 million patients suffering from schizophrenia in the United States.

PROPOSED COURSE

The project described above was initiated during the past year. We will be examining data for promising leads and new directions with the explicit goal of developing better treatments for schizophrenia.

PUBLICATIONS: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02184-01 NS
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biological Tests in Depression		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Alec Roy, M.B., Visiting Associate, Section on Clinical Studies, NS, NIMH		
COOPERATING UNITS (if any) Office of Clinical Director, NIAAA; Sheppard Pratt Hospital, Baltimore, MD		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Clinical Studies		
INSTITUTE AND LOCATION NIMH, ADAMHA, Bethesda, MD 20205		
TOTAL MANYEARS: 10	PROFESSIONAL: 7	OTHER: 3
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The aim of this study is to investigate possible <u>neuroendocrinological</u>, <u>neurotransmitter</u> and <u>membrane marker tests for depression</u>. Depressed patients, free of psychotropic medication for at least 2 weeks, are tested over a 7-day period while on a low monoamine diet. Initially, two 24-hour urine collections are made for urinary MHPG and free cortisol estimations. Blood is drawn for platelet serotonin uptake and [³H]-<u>imipramine binding</u> assays, for plasma MHPG endorphins, <u>norepinephrine</u> and HVA assays. The cold pressor, thyrotropin-releasing hormone (TRH) and dexamethasone suppression (DST) tests are performed. A lumbar puncture is done to measure the neurotransmitter metabolites 5-HIAA, MHPG and HVA.</p> <p>The patients' symptoms are observer-recorded using the Hamilton Rating Scale, the BPRS and the Bunney-Hamburg Scale as well as self-recorded using the POMS and Beck Scales. Premorbid personality is assessed on the Eysenck Personality Questionnaire (EPQ), the Fould's Hostility Questionnaire (HDHQ), self-esteem and obsessional-hysteroid questionnaires. Life events in the antecedent 6-month period are recorded using Paykel's method and premorbid marital and social support assessments are made.</p> <p>In the 8-month period from November, 1982 to June, 1983, we have investigated 46 depressed patients, including 25 recurrent affective disorder patients (17 melancholic in the current episode), 9 dysthymic disorder patients and 12 patients with a major depressive episode. Thus we will be able to examine these tests in 5 groups: recurrent affective disorder (endogenous depression), dysthymic disorder, "neurotic" depression, schizophrenia and normal volunteers. We will be able to examine both the <u>sensitivity</u> and <u>specificity</u> of these biological tests for depression.</p>		
(727)		

OTHER PROFESSIONAL PERSONNEL

Steven M. Paul, M.D.	Chief	NSB	NIMH
David Pickar, M.D.	Chief, Section on Clinical Studies	NSB	NIMH
Owen Wolkowitz, M.D.	Clinical Associate	NSB	NIMH
Allen Doran, M.D.	Clinical Associate	NSB	NIMH
Markku Linnoila, M.D.	Clinical Director		NIAAA
John Boronow, M.D.	Psychiatrist, Sheppard Pratt Hospital Baltimore, MD		

PROJECT DESCRIPTION

The purpose of this project is to investigate possible neuroendocrinological, neurotransmitter and membrane marker tests for depression. Towards this end, we are studying a spectrum of patients with depressive illness in a short-term intensive program on the 4-East Nursing Unit of the NIH Clinical Center. Patients are admitted on referral by outpatient clinicians and are studied following a drug-free period of at least 14 days. Detailed clinical assessments and ratings are completed and will be examined for possible relationships with biological measures. The same biological measures are performed in an age and sex matched group of normal controls.

Methods

I. Ratings:

- A. Hamilton Rating Scale
- B. Brief Psychiatric Rating Scale
- C. Bunney-Hamburg Scale
- D. POMS, BECK and NIMH (self-recorded ratings)
- E. Eysenck Personality Questionnaire
- F. Fould's Hostility Questionnaire
- G. Life Events Inventory (after Paykel's method)

II. Biological Measures:

- A. Urine for MHPG and urinary free cortisol
- B. Plasma for MHPG, norepinephrine, HVA, 5HIAA, β -endorphin, cortisol, ACTH.
- C. Platelet serotonin uptake and [^3H]-imipramine binding assays.
- D. Dexamethasone suppression test (including β -endorphin and ACTH determinations).
- E. TSH response to TRH infusion (including plasma prolactin and cortisol measures).
- F. Lumbar Puncture to provide measures of CSF monoamines and metabolites.
- G. Neuroendocrine and plasma amine response to a cold water stressor test.

MAJOR FINDINGS

We are currently completing the phase of data collection in this project. A total of 46 depressed patients have been studied to date. This group includes 25 recurrent affective disorder patients, 17 with melancholia in the current episode, 9 dysthymic disorder patients and 12 patients with major depressive

episode with no current or prior history of melancholia. The range and richness of clinical data creates a special opportunity for investigating the reported relevance of a number of biological tests for depressive illness. Our goal includes examination for both the sensitivity and specificity of these biological tests for depression.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND TO THE PROGRAM OF THE INSTITUTE

In this project, we have studied patients with a wide range of depressive disorders and normal controls with the rigor of an NIMH Research Ward. Data derived from this project will make an important contribution in the process of establishing potentially useful biological tests in depressive illness. Though other groups of investigators have studied individual of the employed tests, this project is unique in the breadth of study in such a sizable group of depressed patients.

PROPOSED COURSE

Future work in short-term study of depressed patients will focus on patients with a family history of suicide. We are currently developing strategies to assess serotonin function in these individuals.

PUBLICATIONS: None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00167-04 NS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Neurochemical Coding of Brain Pathways Revealed by Autoradiography

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Candace B. Pert, Ph.D., Pharmacologist, NSB, NIMH

COOPERATING UNITS (if any)

Laboratory of Neurophysiology, NIMH

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

4.5

PROFESSIONAL:

4.2

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We continue to study the distribution of brain neurotransmitter or drug receptors by our newly developed autoradiographic method. Chemically coded neuronal pathways can be surmised from the concordance of receptor distributions and autoradiographic tract tracing visualization. A novel radioimmunochemical method enables us to visualize neuropeptides and quantitate them simultaneously at any level of brain. Disappearance of neuropeptide following lesion of putative peptidergic tract provides proof of specific chemically coded pathways.

Project Description:Other Professional Personnel:

Remi Quirion	Guest Worker	NS NIMH
Richard B. Rothman	Guest Worker	NS NIMH
Stafford McLean	Staff Fellow	NS NIMH
Miles Herkenham	Psychologist	LNP NIMH

Objectives:

To map the neuroanatomical distribution of various chemically coded pathways in brain.

Methods Employed:

1. Newly developed in vitro autoradiography--unfixed frozen brain tissue is melted onto slides, incubated in appropriate radioactive ligands to label receptors, washed thoroughly, dried rapidly, fixed with paraformaldehyde vapors, and dipped in radio-sensitive liquid emulsion for traditional autoradiographic workup.
2. Receptor-labelled slides are placed tightly against tritium-sensitive film for two weeks, and then grain density is transformed by computer.
3. Newly developed in vitro radioimmunohistochemical visualization of brain neuropeptides, whereby the binding of primary antibodies against endogenous brain peptides, is visualized by ^{125}I -labelled secondary antibody enabling computer-assisted quantitative analysis of results as well as providing a simple method for visualizing all brain "from a distance" where macroscopic neuroanatomical patterns can be discerned.

Major Findings:

1. Substance P receptors have been mapped for the first time. They share many of the same general locales (within sensory areas, limbic areas, etc.) as opiate receptors, and provide evidence for the notion of substance P and opiate peptides sharing reciprocal modulation perhaps resulting in the perception of pain and pleasure.
2. The cholecystokinin receptor has been visualized for the first time. Great receptor enrichment in the cingulate cortex is noteworthy and interesting in light of the role of this structure in mammalian behaviors.
3. Neurotensin receptors are located on cell bodies and terminals of the dopaminergic projections of rat brain.
4. Lung has beta-adrenergic receptors distributed diffusely and unexpectedly over all tissue surfaces.
5. An opiate projection exists from cell bodies in the bed nucleus of the stria terminalis and projects to the habenula. This has been demonstrated by the use of radioimmunohistochemical visualization.

6. The pattern of phencyclidine (angel dust) receptor distribution suggested a pattern reminiscent of other neuropeptide receptors. Indeed, we have prepared peptide extracts of hippocampus, where angel dust receptors are densest, which have phencyclidine-like activity in a binding assay and two behavioral assays.

Significance to Biomedical Research and Program of the Institute:

Pinpointing neurochemically coded tracts by this defined neuroanatomical procedure will enable us to perform lesioning and drug mimicry experiments to determine the functional significance of each newly discovered pathway. The method can be used on human brain, and ultimately should give information about the contribution of various neurochemically coded tracts to pathology.

Proposed Course

As we achieve visualizations of more neuropeptides and their receptors, we are enabled to make general statements about the role of neuropeptides in brain. Analysis up to this point suggests that neuropeptides, based upon their neuroanatomical localization, play a role in the biochemistry of emotions, i.e. the biasing of sensory input for determining which perceptions have the highest priority for being acted upon.

Publications

1. Quirion, R., Bowen, W.D., Herkenham, M., and Pert, C.B. Visualization and solubilization of rat brain opiate receptors with a kappa ligand selectivity pattern. Cell. Mol. Neurobiol. 2: 333-346, 1982.
2. Quirion, R., Hammer, R.P., Jr., Herkenham, M. and Pert, C.B. The phencyclidine (angel dust/sigma "opiate" receptor. In Proceedings of the 43rd Annual Scientific Meeting of the Committee on Problems of Drug Dependence, 1982, 178-183.
3. Pert, C.B., and Quirion, R. The phencyclidine receptor. Trends Pharmacol. Sci. 4: 12-13, 1983.
4. Altura, B.T., Quirion, R., Pert, C.B. and Altura, B.M. Phencyclidine (angel dust) analogs and sigma opiate benzomorphans cause cerebral arterial spasm. Proc. Natl. Acad. Sci. USA 80: 865-869, 1983.
5. Quirion, R., Gaudreau, P., St-Pierre, S., Rioux, F., and Pert, C.B. Autoradiographic distribution of [^3H]neurotensin receptors in rat brain: visualization by tritium-sensitive film. Peptides 3: 757-769, 1983.
6. Gaudreau, P., Quirion, R., St-Pierre, S., and Pert, C.B. Tritium-sensitive film autoradiography of ^3H -cholecystokinin-5/pentagastrin receptors in rat brain. Eur. J. Pharmacol. 87: 103-104, 1983.
7. Quirion, R., Shults, C.W., Moody, T.W., Pert, C.B., Chase, T.N., and O'Donohue, T.L. Autoradiographic distribution of substance P receptors in rat central nervous system. Nature 303: 714-716, 1983

8. Quirion, R., Shults, C.W., Moody, T.W., Wolf, S.S., Jensen, R.T., Pert, C.B., Chase, T.N., and O'Donohue, T.L. Autoradiographic localization of substance P (SP) receptors in rat brain: a comparison of binding properties of [^3H]SP, [^{125}I]SP, and [^{125}I]physalaemin. In Skrabranek, P. and Powell, D. (Eds.): Substance P. Dublin, Ireland, Boole Press, 1983, pp. 55-56.
9. Lewis, M.E., Pert, A., Pert, C.B., and Herkenham, M. Opiate receptor localization in rat cerebral cortex. J. Comp. Neurol. 216: 339-358, 1983.
10. McLean, S., Skirboll, L., and Pert, C.B. Opiatergic projection from the bed nucleus to the habenula: demonstration by a novel radioimmunochemical method. Brain Res., in press.
11. Rothman, R.B., Herkenham, M., Pert, C.B., Liang, T., and Cascieri, M.A. Visualization of rat brain receptors for the neuropeptide SP. Proc. Natl. Acad. Sci. USA, in press.
12. Gaudreau, P., Quirion, St-Pierre, S., and Pert, C.B. Characterization and visualization of cholecystokinin receptors in rat brain using [^3H]peptagastrin. Peptides, in press.
13. Gaudreau, P., Morell, J.L., St-Pierre, S., Quirion, R., and Pert, C.B. Cholecystokinin octapeptide fragments: synthesis and structure-activity relationship. In Hruby, V. and Rich, D. (Eds.): Peptides: Proceedings of the Eighth American Peptide Symposium. Rockford, Illinois, Pierce Chemical, in press.
14. O'Donohue, T.L., Quirion, R., Pert, C.B., Pert, A., French, E.D., and Everist, H. Evidence for an endogenous central nervous system ligand to the phencyclidine receptor. In Hruby, V. and Rich, D. (Eds.): Peptides: Proceedings of the Eighth American Peptide Symposium. Rockford, Illinois, Pierce Chemical, in press.
15. Quirion, R., O'Donohue, T.L., Everist, H., Pert, A., and Pert, C.B. Phencyclidine receptors and possible existence of an endogenous ligand. In Kamenka, J.M., Domino, E.F., and Geneste, P. (Eds.): Phencyclidine and Related Arylcyclohexylamines: Present and Future Applications. Ann Arbor, Michigan, NPP Books, in press.
16. Quirion, R., Herkenham, M., O'Donohue, T.L., and Pert, C.B. Autoradiographic localization of ^3H -ethylketocyclazocine binding sites in rat brain. In Cros, J. and Roncucci, R. (Eds.): "Kappa Opiate" Receptors. Paris, France, Quo Vadis, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00169-03 NS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Allosteric Receptor Modulation and Altered Sensitivity States

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Candace B. Pert, Ph.D., Pharmacologist, NSB, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Brain Biochemistry

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

Work on this project has been temporarily delayed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02182-01 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Toward the Visualization of Opiate Receptors in Living Humans		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Candace B. Pert, Ph.D., Pharmacologist, NSB, NIMH		
COOPERATING UNITS <i>(if any)</i> Laboratory of Chemistry, NIADDK; Nuclear Medicine Branch, Clinical Center		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Brain Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.6	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> The distribution of positron emitting substances in brain can be followed by <u>positron emission tomography</u> (PET). We are developing ¹⁸ F-labelled high affinity opiate drugs to be injected into living humans for the visualization of <u>opiate receptor patterns in vivo</u> . It will be interesting to determine whether <u>opiate receptor distribution patterns</u> in cortex change as a function of attention and emotional states.		

Other Professional Personnel:

Terrence Burke	Pharmacologist	LC	NIADDK
Michael Channing	Physician	NM	CC
Robert Kessler	Physician	NM	CC
Ronald Manning	Physician	NM	CC
Kenner Rice	Pharmacologist	LC	NIADDK
Uwe Kurt Schumacher	Chemist	NS	NIMH

Project Description:Objectives:

To demonstrate gradients of opiate receptor density in the cortex of living humans. To examine whether differences in these gradients exist as a function of emotional state or attentional processes.

Methods Employed:

PET Scan--using newly developed ^{18}F -labelled opiate analogs.

Major Finding:

We managed to affix a fluoride moiety to phenazocine, a potent opiate analgesic without loosing very much affinity for opiate receptors. This fluoro-opiate derivative would be suitable for in vivo injections for visualizing receptors.

Significance to Biomedical Research and Program of the Institute:

The notion that alterations in mood are a function of oscillations in neurotransmitter receptor sensitivity is perhaps the most exciting new lead in attempting to understand the causes of mental illness. Other leads in this institute point to the relevance of extent of cortical participation as a critical factor in psychiatric disease.

Proposed Course:

We plan to characterize Fluorophan by obtaining radiolabelled compound and injecting it in vivo into rats and monkeys to demonstrate that it binds to opiate receptors as previously visualized. Furthermore, we continue to develop more fluoro-labelled opiates, always seeking a very simple chemical (one-step synthesis) in order to do the rapid chemistry necessary for working with the fleeting ^{18}F isotope.

Publications:

1. Rice, K.C., Konicki, P.E., Quirion, R., Burke, T.R. and Pert, C.B. Synthesis and pharmacological characterization of (\pm)-5,9 α -dimethyl-2-[2-(4-fluorophenyl)ethyl]-2'-hydroxy-6,7 benzomorphan (Fluorophan). A ligand suitable for visualization of opiate receptors in vivo. J. Med. Chem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02183-01 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Is Schizophrenia an Autoimmune Neuropeptide Receptor Disease?		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Candace B. Pert, Ph.D., Pharmacologist, NSB, NIMH		
COOPERATING UNITS (if any) Uniformed Services University of the Health Sciences; Laboratory of Psychology and Psychopathology		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Brain Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL:	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The notion that <u>schizophrenia</u> has an important autoimmune component has been around for several decades but has not previously been subjected to analysis by the most sensitive, modern techniques. We have developed a simple sensitive assay for detecting <u>antibodies</u> directed against human brain found in sera of schizophrenic patients and controls. We are now exploring the frequency of these antibodies in schizophrenics vs. controls and characterizing the molecular properties of the <u>brain antigens</u> involved and their distribution by <u>visualization</u> in rodent and human brain.		

Other Professional Personnel:

Richard Weber
Lynn DeLisi

Immunologist
Psychiatrist

USUHS
LPP NIMH

Project Description:Objectives:

1. To develop a simple assay for demonstrating brain-directed autoantibodies in schizophrenic sera.
2. To demonstrate the molecular properties of these brain antigens and their distribution in brain tissue.
3. To explore the possibility that the antigens are cell surface neuropeptide receptors which mediate the biochemistry of emotion.

Methods Employed:

A novel filtration and centrifugation assay for detecting brain antigens in sera and the new (McLean *et al*, Brain Res., in press) method for visualizing antibody distribution patterns in brain.

Major Findings:

In collaboration with Dr. Weber, over twenty experiments were performed for the purpose of optimizing the conditions of the antibody detection assay. In an early blind experiment, six of the Clinical Center 4-East ward acute schizophrenics' and controls' sera were examined. The two highest numbers in the assay belonged to the two sickest patients. We utilized the sera from these two patients vs. two controls in every experiment as we worked on optimization. The assay appears to sensitively and repeatedly demonstrate differences in these sera and another patient whose serum was recently screened by Dr. DeLisi. The new patient's serum level seems elevated even after repeated blood sample withdrawals over a period of one year. We know the history of this area and are proceeding cautiously.

Significance to Biomedical Research and Program of the Institute:

Schizophrenia is a crippling psychiatric disease which affects one percent of the general population. A complete, convincing understanding of its etiology would almost certainly lead to better therapeutic strategies and would place this psychiatric illness in a more "normal" context with other diseases of the body.

Proposed Course:

We must now collect a large number of determinations on many sera to describe the incidence in normal and schizophrenic sera as well as correlating psychotic symptoms with antibody titers over time with one patient. We plan and to perform appropriate controls for neuroleptic drug treatment. We plan future experiments to demonstrate that the incidence of antibodies directed against

brain are much higher in schizophrenics--and perhaps certain subtypes--than in normal controls, and that this incidence is not due to neuroleptic drug treatment.

Publications:

In preparation

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02185-01 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) What is the Etiology of Small Cell Carcinoma of the Lung?		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Candace B. Pert, Ph.D., Pharmacologist, NSB, NIMH		
COOPERATING UNITS (if any) Laboratory of Microbiology and Immunology, NIDR		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Brain Biochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.9	PROFESSIONAL: 0.5	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Previous work from this laboratory has demonstrated that small cell carcinoma of the lung is characterized by the presence of the neuropeptide bombesin, which is also excreted into the serum of patients during the terminal stages of the illness. The assumption that small cell "lung cancer" arises as a primary tumor from lung appears without experimental support--the true origin of this disease is in haemopoetic cells, arising from bone marrow.</p>		

Other Professional Personnel:

Michael Ruff

Immunologist

LMI NIDR

Project Description:Objectives:

To demonstrate the true etiology of small cell carcinoma of the lung in order to gain information for intelligent therapeutic intervention. To examine the role of bombesin and other neuropeptides as regulators of the body's immune system.

Methods Employed:

1. Radioimmunoassay
2. Effects of bombesin and other neuropeptides on cell culture

Major Findings

1. Small cells contain MAC-1, an antibody which characterizes macrophages on their cell surface, along with other macrophage cell surface markers, suggesting that the lung origin of this cancer has been misconstrued.
2. The neuropeptide bombesin appears to be chemotactic for haemopoietic cells which become blood elements like macrophages--thus small cell carcinoma of the lung appears to be a primitive macrophage "stuck" in a replicating mode but sharing some cell surface properties of mature macrophages.

Significance to Biomedical Research and Program of the Institute

One hundred thousand people contract the viral form of lung cancer called small cell each year. Ninety percent of them die within six months. All therapeutic strategies up to this point have proved worthless, prolonging life by a mean of three to four months. An understanding of the true etiology of the disease would lead to therapeutic strategies aimed at utilizing the body's own immune mechanisms to stop the replication of the primitive, out-of-control macrophage which we call oat cell carcinoma of the lung. While the study of lung cancer at first may not appear to be directly applicable to the mission of the Institute, an interesting side effect of the terminal stages of oat cell disease is severe psychotomimetic symptoms which may be due to the activation of brain peptide receptors with the neurocirculatory products of the lung cancer cell.

Proposed Course:

We plan immunohistochemical demonstration of macrophage cell surface markers lighting up tumorous lung but not normal lung. Further investigations of the role of neuropeptides in the immune system are planned.

Publications:

1. Moody, T.W., Pert, C.B., Gazdar, A.F., Carney, D.N. and Minna, J.D. High levels of intracellular bombesin characterize human small-cell lung carcinoma. Science 214: 1246-1248, 1981.

2. Pert, C.B. and Schumacher, U.K. Elevated plasma bombesin in patients with extensive small cell carcinoma of the lung. Letter to the Editor, Lancet, February 27, 1982: 509.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00117-08 NS
PERIOD COVERED <u>October 1, 1982 through September 30, 1983</u>		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>α-Adrenergic and Prostaglandin Receptors in Human Blood Elements</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <u>Marian S. Kafka, Physiologist, NSB, NIMH</u>		
COOPERATING UNITS (if any) <u>Clinical Studies Section, NSB; Clinical Neuropharmacology, NIMH; Section on Psychogenetics, BPB; Unit on Anxiety and and Affective Disorders, CPB</u>		
LAB/BRANCH <u>Clinical Neuroscience Branch</u>		
SECTION <u>Section on Molecular Pharmacology</u>		
INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Bethesda, Maryland 20205</u>		
TOTAL MANYEARS: <u>0.5</u>	PROFESSIONAL: <u>0</u>	OTHER: <u>0</u>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>α-Adrenergic receptors and prostaglandin receptors are being studied in human blood cell preparations. The ability of adrenergic agonists to inhibit adenylate cyclase is used as a measure of the α_2-receptors' biological function. Binding of tritiated α-adrenergic receptor antagonists to membrane receptors on platelets is being measured. These correlative measures are used to assess α-adrenergic physiological function in normal human beings, patients with various psychiatric disorders, and patients receiving different psychopharmacologic agents.</u>		

Other Professional Personnel:

John Nurnberger	Psychiatrist	BPB NIMH
Alec Roy	Psychiatrist	NS NIMH
Larry Siever	Psychiatrist	CN NIMH
Thomas Uhde	Psychiatrist	CNB NIMH

Project Description:Objectives

- (1) To study alterations in α adrenergic receptor number and function in psychiatric diseases and other pathological conditions characterized by altered adrenergic transmission. To measure changes in receptor number and function with different treatment modalities.
- (2) To provide an experimental model for the study of drug-induced or physiologically-induced changes in central receptor sensitivity in man.
- (3) To understand the cellular events connecting receptors, occupied by their agonists, with the activity of the adenylate cyclase enzyme complex and subsequent physiological events.

Methods Employed:

To measure α -adrenergic receptors, human platelets are prepared from a fresh blood sample. An aliquot of the platelets is washed and resuspended for measurement of cyclic AMP production. The remainder of the platelets are homogenized to yield a membrane preparation which is washed and resuspended for the measurement of tritiated α -adrenergic antagonist binding.

Major Findings

Chronic clorgyline administration does not change the number of α_2 -receptors or prostaglandin E_1 (PGE_1)-stimulated cyclic AMP (cAMP) production in platelets from depressed patients, whereas both basal cAMP production and plasma norepinephrine (NE) concentration were decreased.

Significance to Biomedical Research and to the Program of the Institute

The results obtained from these experiments are directly applicable to an assessment of receptor function in human disease states, and to monitoring the physiological effects of various drug treatments. If peripheral α_2 -adrenergic receptors are related to, or can serve as models for the central nervous system α_2 -receptors, valuable information about the function of these central receptors in psychiatric diseases may be obtained.

It is possible that α_2 -adrenergic receptor function in central and peripheral nervous systems is similar to that measured in platelets. Platelet α_2 -receptor number and PGE_1 -stimulated cAMP production are unchanged with chronic clorgyline treatment. Animal studies, on the other hand, suggest that clorgyline decreases the number of brain α_2 -receptors. If the brains in man and rat are similar (and central and peripheral α_2 -receptors are similar), the action of clorgyline in reducing the number of α_2 -receptors may represent a specific

central adaptation to the administration of the drug rather than a direct action on the α_2 -receptor complex. A comparison of brain and platelet changes with drug administration may help to differentiate the actions of drugs which are directly on the receptor molecule and those which are indirect, i.e., through receptors or receptor-mediated processes. Such data can provide information important in the interpretation of drug action.

Proposed Course

To continue the examination of mechanisms of information transfer between α_2 -receptors and the adenylate cyclase complex. To measure in greater detail α_2 -adrenergic receptor function in platelets from patients with schizophrenia and affective illness. To investigate whether any changes measured are trait- or state-dependent, whether they are correlates of unipolar or bipolar illness, and whether they may be altered by some treatment modalities.

Publications

1. Kafka, M.S. and van Kammen, D.P.: Alpha-adrenergic receptor function in schizophrenia: receptor number, cyclic AMP production, adenylate cyclase activity, and the effect of drugs. Arch. Gen. Psychiatry 40: 264-270, 1983.
2. Siever, L.J., Uhde, T.W., Jimmerson, D.C., Kafka, M.S., Lake, C.R., Targum, S., and Murphy, D.L.: Clinical studies of monoamine receptors in affective disorders and receptor changes with antidepressant treatment. In Progress in Neuro-Psychopharmacology and Biological Psychiatry, in press.
3. Siever, L.J., Kaye, W.H., Jimmerson, D.C., Kafka, M.S., Lake, C.R., Targum, S., and Murphy, D.L.: Abnormalities in the primary affective disorders compared to other tricyclic-responsive disorders. Psychopharm. Bull., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00159-04 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotransmitter Receptors in the Nervous System		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Marian S. Kafka, Physiologist, NSB, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Rat brain neurotransmitter receptors are being studied. There are <u>circadian rhythms in neurotransmitter receptors</u> in relatively <u>discrete brain regions</u> , as well as in whole "forebrain." In cerebral <u>cortical adrenergic receptors</u> there are <u>circadian rhythms</u> which are related to <u>circadian rhythms in a functional response to receptor activation</u> , such as the production of cyclic AMP (cAMP).		

Project Description:Objectives:

- (1) To develop methods to measure the presence of neurotransmitter receptors in the nervous system.
- (2) To measure whether alterations in receptor number accompany alterations in function in the central nervous system.
- (3) To assess whether there are rhythms in functionally significant responses to receptor activation.

Methods Employed:

The specific binding of tritiated ligands to membranes prepared from rat brains is used to measure rhythmic changes in receptor binding in rats sacrificed at intervals over a 24-hour period. The α_1 -receptor is measured by the binding of ^3H -WB4101 or ^3H -prazosin; the β -adrenergic receptor, by ^3H -dihydroalprenolol; the muscarinic acetylcholine receptor by ^3H -QNB; the benzodiazepine receptor by ^3H -flunitrazepam; and the α_2 -receptor by ^3H -para-aminoclonidine. Methods to measure binding in very small tissue samples were devised.

Major Findings:

- (1) There are circadian rhythms in receptors not only in the large regions previously measured ("forebrain," i.e., the brain rostral to the cerebellum but without the striata), but in smaller regions, e.g., the cerebral cortex, hypothalamus, and the pons.
- (2) There is a circadian rhythm in an intracellular biochemical response to α_1 - and β -adrenergic receptor occupancy, viz. the norepinephrine (NE)-stimulated cAMP production in cerebral cortical slices. The rhythms in cAMP production resemble the rhythms in α_1 - and β -receptors.

Significance to Biomedical Research and the Program of the Institute:

Previous work documented the existence of circadian rhythms in neurotransmitter receptor density. These rhythms changed with chronic psychoactive drug administration. The new studies examine whether circadian rhythms in receptors are functionally significant. In vitro, the circadian rhythm in the number of α_1 - and β -adrenergic receptors stimulated by NE can regulate the circadian rhythm in cAMP production. Perhaps the number of adrenergic receptors stimulated by NE modulates the magnitude of cAMP production in situ. As the intraneuronal cAMP concentration is thought to act as a second messenger, regulating the phosphorylation and activation of cellular proteins, circadian rhythmic changes in neuronal cAMP production could have a profound effect on neuronal activity and neuronal transmission across the day.

Proposed Course:

Whether in situ there are circadian rhythms in neurotransmitter turnover, receptors, and cAMP concentration, and, if so, what their relationships are, is being

investigated in small brain regions. The relationship between rat brain circadian receptor rhythms and rat electroencephalographic patterns is being investigated.

Publications:

1. Wirz-Justice, A., Kafka, M.S., Naber, D., Marangos, P.J., Campbell, I.C., and Wehr, T.A.: Clorgyline modifies circadian neurotransmitter receptor rhythms. Brain Res. 241: 115-122, 1982.
2. O'Donohue, T.L., Wirz-Justice, A., Kafka, M.S., Naber, D., Campbell, I.C., and Wehr, T.A.: Effect of chronic lithium, clorgyline, imipramine, fluphenazine, and constant darkness on alpha-melanotropin content and circadian rhythms in rat brain. Eur. J. Pharmacol. 85: 1-7, 1982.
3. Naber, D., Wirz-Justice, A., and Kafka, M.S.: Chronic fluphenazine treatment modified circadian rhythms of neurotransmitter receptor binding in rat brain. J. Neural. Transm. 55: 277-288, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER 701 MH 00179-02 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Morphological and Functional Aspects of Peptides in Mammalian Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Lana Skirboll, Ph.D., Pharmacologist, NSB, NIMH; Daniel Hommer, M.D., Psychiatrist, NSB, NIMH		
COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; Pharmacology Department, Georgetown University		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Using <u>immunohistochemical</u> techniques in combination with <u>retrograde tracing</u> , we have most recently extended our findings on the coexistence of <u>cholecystokinin</u> (CCK) and <u>dopamine</u> (DA) to include the cat, and have shown that CCK coexists with <u>substance P</u> (SP) in the periaqueductal gray in this species as well. Descending supraspinal rat pathways to the spinal cord have been explored more extensively to include <u>enkephalin</u> , <u>SP</u> , and <u>somatostatin</u> . Evidence that a tri-transmitter system <u>SP-5HT-TRH</u> innervates the spinal cord has been explored in more detail by examining the next step in the pathway. Dye injections into the diaphragm of the cat combined with immunocytochemistry has revealed that the ventral motor neurons are innervated by these three putative transmitter substances, and studies are presently being planned to explore this innervation physiologically using <u>iontophoretic</u> application of peptides while monitoring motor nucleus activity. In addition, peptide innervation of the paraventricular nucleus of the hypothalamus is being explored preliminary to electrophysiological studies of the role of CRH and adrenaline on ACTH release. Finally, methodologically we have developed a method for <u>radioimmunocytochemistry</u> which allows us to use autoradiographic techniques to visualize the antibody antigen complex. This permits us to compare putative transmitter innervation areas with autoradiographic analysis of receptor density as well as comparing patterns of <u>immunoreactive peptide terminals</u> which are suspected examples of coexistence.		

Other Professional Personnel:

Richard Gillis, Ph.D.
 Stafford McLean, Ph.D.
 Eva Mezey, Ph.D.
 Candace B. Pert, Ph.D.

Pharmacologist
 Staff Fellow
 Guest Worker
 Pharmacologist

Georgetown University
 NS NIMH
 LCS NIMH
 NS NIMH

Project Description:Objectives

The realization of the remarkable number of peptide neurotransmitters in the mammalian central nervous system has recently made it important to identify neuron populations on the basis of their chemical content. Isolation of neuropeptides and subsequent production of antisera to these compounds has led to the visualization of one and sometimes more transmitter(s) in single neurons in brain. This laboratory has been involved in the morphological and functional aspects of peptide transmitters in mammalian brain. Using immunohistochemical and electrophysiological techniques we have primarily explored the phenomena of coexistence (i.e., more than one putative transmitter substance in a single neuron). Immunocytochemical studies have been extended by using retrograde tracing techniques in which pathways can be traced on the basis of chemical transmitter.

Methods Employed

During the past year our laboratory has been using immunocytochemical techniques to further elucidate transmitter specific pathways using a combination of immunocytochemistry and retrograde tracing of fluorescent dyes. In general, animals are first treated with colchicine to block axonal transport of peptide and thus accumulate antigen in the cell body, followed by antisera conjugated to a fluorescence molecule to allow visualization of transmitter-specific fluorescence of single cells under the fluorescence microscope. Studies in which peptide pathways were being explored were preceded by dye injected into proposed nerve terminal areas. The dye was taken up into axons and transported back into the cells of origin and visualized in the fluorescence scope. Once the dye is viewed, these same sections can subsequently be stained for immunocytochemical procedures as described above, thus permitting transmitter-specific mapping of neuroprojections.

Major Findings

Using these techniques we have extended last year's findings that CCK and SP coexist in neurons projecting to the spinal cord in the rat to evidence that similar projections occur in the cat. More extensive examination of supraspinal projections in the rat has revealed that SP neurons in the raphe magnus project to the ventral horn of the spinal cord. We have also observed that an enkephalin immunoreactive system gives rise to descending axons with cell bodies located dorso-lateral to the pyramidal tracts. Evidence has also been obtained that somatostatin immunoreactive cell bodies in the intercommissuralis and the AI/CI cell group also both project to the spinal cord. Finally, these studies of peptide projections to the spinal cord have revealed that the coexisting serotonin-thyrotropin stimulating hormone, SP (5-HT-TRH-SP) cell bodies of the

medullary raphe project to the ventral horn of the spinal cord of the rat and cat.

Having established that cells in the A1/C1 group project to the spinal cord, we sought to examine those neurons which innervate this catecholamine group. Dye was injected into A1/C1 area in the same manner as described above. Fluorescent cells were found in the paraventricular nucleus of the hypothalamus (PVN). By combining these retrograde labelling studies with immunocytochemistry, preliminary evidence has been obtained that there are vasopressin, neurotensin, CCK and TRH cells in the PVN which project to the A1/C1 area of the brainstem.

In another series of studies we have drawn on information from our work on supraspinal projections to the spinal cord. We found that there is a tri-transmitter system (5HT-SP-TRH) which projects to the ventral horn of the spinal cord. Since the ventral horn of the spinal cord is rich in motor neurons, we chose to examine one motor system in light of its innervation. Retrograde tracing in combination with immunocytochemistry was again employed to identify the putative neurotransmitters in the phrenic motor nucleus of the cat. Fast blue dye was injected bilaterally into the diaphragm of the cat, resulting in fluorescent-labelled cells in the fifth cervical segment of the ventral horn. Immunocytochemical studies revealed that SP-, TRH-, and 5HT-containing fibers were in close proximity to both phrenic motor neurons and its dendrites.

Projected Course

Since the PVN is an area of the hypothalamus which is involved in several vegetative functions through which the integration of hypothalamic endocrine and autonomic responses to visceral stimuli might be mediated, it will be of interest to continue to examine both the morphology and function of this system. Initially we plan to determine if there is a feed-back system from the A1/C1 system to the PVN by using the retrograde tracing-immunocytochemical technique. It is presently thought that peptides from the PVN neurons are released into the portal vessels to influence the release of ACTH from the pituitary.

Secondly, with regard to the phrenic motor nucleus, it has been suggested that 5HT, SP and TRH are important neurotransmitters and/or neuromodulators involved in the central control of respiration, we plan to extend these studies to a more physiological evaluation of coexistence. Studies are planned to record from the phrenic motor neuron and using iontophoretic application.

Finally, since fluorescence or peroxidase-antiperoxidase (PAP) immunohistochemistry provide morphological detail but do not allow an easy assessment of peptide distribution (as they require a microscope with its narrow field of view), we have recently developed a radioimmunohistochemical technique. This technique uses a radiolabeled antibody that allows us to use autoradiographic techniques to visualize the antibody-antigen complex. Cryostat-cut sections are slide-mounted and then incubated with 1° antibody. Sixteen to 36 hours later the sections are rinsed and the ¹²⁵I-labelled secondary antibody, made against the 1° antibody, is applied. The slides are then apposed to LKB Ultrafilm or dipped in nuclear back emulsion. Preliminary findings using this technique include evidence that lesions of the bed nucleus of the stria terminalis result in a loss of immunoreactive material in the ipsilateral habenula, as measured by optical density of the film. In addition, examination of the

distribution of SP and enkephalin in adjacent sections reveals a dramatic concordance in their distribution.

Finally, radioimmunochemistry can be applied to several problems: (1) comparisons of published autoradiographic maps of receptors and immunohistochemical maps of the ligands do not show a perfect concordance. We are currently investigating this perplexing situation using autoradiography to map both receptors and their putative ligands. Our goal is to do receptor autoradiography and radioimmunochemistry in adjacent sections from the same brain, thus making a direct comparison of receptor and ligand distribution; (2) radioimmunochemistry and the peroxidase-anti peroxidase immunohistochemical method are comparable at both the light microscopic and the EM level of analysis. We plan to investigate the coexistence of peptides in the same neuron, and at the EM level investigate the ultrastructural localization in the cell body, and (3) quantification of immunocytochemistry has been an elusive goal. The use of radioimmunocytochemistry allows the use of computer-assisted programs for quantitative autoradiography to start the process of quantifying immunocytochemistry.

Significance to Biomedical Research

Identification of more and more peptide putative transmitters in brain has permitted elucidation of a more refined network in mammalian nervous tissue. The use of the immunohistochemical technique which allows the tracing of chemical specific pathways in brain is only limited by the ability to raise antisera to a specific antigen. Such techniques especially used in common with retrograde tracing procedures will continue to reveal more about functional transmitter pathways in brain. Furthermore, the development of radioimmunocytochemical techniques will lead to a coincidental mapping of both putative transmitter substances and receptors. This may lead to the development of site-specific drugs for the treatment of both neurological and psychiatric illness.

Publications

1. Hokfelt, T., Skirboll, L., Dalsgaard, C.J., Johansson, O., Lundberg, J., Norell, G. and Jansco, G.: Peptide neurons in the spinal cord with special reference to descending system. In Sjolund, B. and Bjorklund, A. (Eds.): Brainstem Control of Spinal Mechanisms. Amsterdam, Elsevier/North-Holland, 1982, pp. 89-117.
2. Vincent, S.R., Johansson, O., Skirboll, L.R. and Hokfelt, T. Co-existence of somatostatin and avian pancreatic polypeptide-like immunoreactivities in striatal neurons which are selectively stained for NADPH-diaphrase activity. In Costa, E. and Trabucchi, M. (Eds.): Regulatory Peptides: From Molecular Biology to Function. New York, Raven Press, 1982, pp. 453-462.
3. Hokfelt, T., Skirboll, L., Lundberg, L., Dalsgaard, C.J., Johansson, O., Pernon, B. and Jansco, G.: Neuropeptides and pain pathways. In Advances in Pain Research, vol. 5. Raven Press, New York, 1983, pp. 227-246.
4. Hokfelt, T., Skagerberg, G., Skirboll, L.R. and Bjorklund, A.: Combination of retrograde tracing and neurotransmitter histochemistry. In Bjorklund, A. and Hokfelt, T. (Eds.): Handbook of Chemical Neuroanatomy, vol. 1. Amsterdam, Elsevier/North-Holland, 1983, pp. 182-196.

5. Skirboll, L.R., Hokfelt, T., Dockray, G., Rehfield, J., Brownstein, M. and Cuello, C.: Evidence for periaqueductal cholecystokinin-substance P neurons projecting to the spinal cord. J. Neurosci. 6: 1151-1157, 1983.
6. Vincent, S.R., Hokfelt, T., Skirboll, L.R. and Wu, J-Y.: Hypothalamic GABA neurons project to the neocortex. Science 220: 1309-1311, 1983.
7. Hokfelt, T., Lundberg, J., Skirboll, J.R., Johansson, O., Schulzberg, M. and Vincent, S.R. (Eds.): Co-Existence of Classical Transmitters and Peptides in Neurons. British Pharmacology Society, John Wiley, New York, in press.
8. Skirboll, L.R., Hokfelt, T., Kuypers, H.G.J.M., Bentovoglio, M., Catsman-Berrevoets, C., Goldstein, M., Steinbusch, A., Verhofstad, A., Steinbusch, J., Jeffcoate, S., Phillipson, O., Dockray, G., Brownstein, M. and Norell, G.: A method for specific transmitter identification of retrogradely labeled neurons: immunocytochemistry combined with fluorescence tracing. Neuroscience, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02177-01 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Behavioral Functions of Neuropeptides		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Jacqueline N. Crawley, Ph.D., NSB, NIMH		
COOPERATING UNITS <i>(if any)</i> Section on Brain Biochemistry, NSB, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> The past decade has witnessed the discovery of thirty or more peptides localized in mammalian central neurons. Cholecystokinin (CCK) in a gut peptide recently found in microgram concentrations in cerebral cortex, limbic structures, and spinal cord. Cholecystokinin appears to coexist with <u>dopamine</u> in the mesolimbic pathway from ventral tegmentum to nucleus accumbens and olfactory tubercle. Behavioral techniques provide useful tools for elucidating the mechanism of neuromodulation of a known neurotransmitter by a peptide. Understanding of CCK modulation of dopamine may lead to the development of more specific and effective antipsychotic treatments.		

Other Professional Personnel:

Lana Skirboll	Staff Fellow	NSB NIMH
Daniel Hommer	Staff Psychiatrist	NSB NIMH
Pierrette Gaudreau	Guest Worker	NSB NIMH

Project Description:Objectives:

Investigation of the neuromodulatory role of cholecystokinin (CCK) on dopamine-mediated behaviors. Tracing the cholecystokinin feedback loop relaying sensory information on feeding and satiety from the gut to the brain.

Methods Employed:

Stereotypy and locomotor activity, exploratory behaviors, food consumption measurements, in mice and rats. Aseptic stereotaxic implantation of indwelling cannulas into brain nuclei of rats. Microinfusion of peptides and drugs into brain nuclei. Histological verification of injection site (Skirboll).

Major Findings:

CCKg-sulfate injected directly into the nucleus accumbens of rats potentiated the behavioral actions of apomorphine. CCK in the dose range of 400 microgram to 40 nanograms increased the degree of stereotypy induced by systemic apomorphine. CCK alone did not induce stereotypy over the dose range of 40 pg to 4 µg. CCK, therefore, appears to facilitate the actions of dopaminergic agonists. These findings support the concept of peptide modulation of transmitter function. In the case of CCK-DA coexistence in the meso-limbic dopamine system, CCK appears to sensitize or synergize dopamine-mediated behaviors without having any intrinsic actions when administered alone.

CCK has been implicated as a signal for feeding satiety. Peripheral CCK receptors in the digestive tract have been shown to mediate food consumption, reducing total food intake in fasted rats, mice, sheep, pigs, monkeys, and humans. We have shown that a variety of behaviors associated with the behavioral state of satiety are mimicked by intraperitoneally injected CCKg-sulfate. These behaviors include reduced exploration of a novel environment, reduced approaches to a novel object, reduced social interactions, and increased periods of behavioral inactivity in the corners of an open field environment. Recent experiments characterize the CCK syndrome as significantly different from behaviors evoked by bombesin, another gut peptide which reduces food consumption, and lithium chloride, a control for aversive internal sensations. The CCK syndrome of reduced exploration was found to be a function of accelerated habituation to the novelty of the test environment, rather than a sedating effect.

The molecular structure of the active site of CCKg-sulfate necessary to induce the satiety syndrome is unknown. Using the exploratory paradigm in

mice as a bioassay, CCK fragments are being tested for behavioral activity. N-terminal and C-terminal CCK 8,7,6,5,4,3,2, fragments have been synthesized (Gaudreau). Fragments with six or less amino acids appear to have greatly reduced behavioral effects. Since the pancreatic CCK receptor binds CCK 8,7,6,5, and 4, and these several fragments induce pancreatic amylase secretion, behavioral potency appears to differ significantly from digestive gland potency. These preliminary data suggest that the effects of CCK on satiety may be mediated by a peripheral site other than the pancreatic CCK receptor. One candidate for the behavioral site is the CCK receptor recently discovered on the vagus nerve. These two receptors may therefore have different properties.

Proposed Course of Project:

Investigation of the mechanism underlying CCK modulation of dopamine is the major area for future research. This question will be addressed at the level of presynaptic versus postsynaptic receptors, by behavioral analysis of CCK and dopamine injected into the ventral tegmentum versus the nucleus accumbens. CCK antagonists under development (R. Jensen, NIADDK) and proglumide, a non-peptide antagonist of the peripheral CCK receptor, will be administered in conjunction with dopaminergic agonists and antagonists. Supersensitivity to CCK after chronic neuroleptic blockade of dopamine receptors will also be tested.

Significance to Biomedical Research and the Program of the Institute:

Several neuropeptides have been found to co-localize with known transmitters. Their discovery in the past few years destroyed the dogma of one-neuron-one-transmitter. The functional significance of co-existing neuromodulators is one of the major basic research questions in neuroscience today. The clinical significance of this research lies in the relationship of CCK to dopamine in the etiology of schizophrenia. Cholecystokinin and dopamine co-exist in the mesolimbic pathway, considered a major site of action of antipsychotics. CCK agonists and antagonists may mimic, potentiate, or augment the actions of a neuroleptic. Clinical trials of CCK are now in progress in our branch (Homer).

Publications:

1. Crawley, J.N. and Beinfeld, M.C. Rapid development of tolerance to the behavioral actions of cholecystokinin. Nature 302: 703-706, 1983.
2. Crawley, J.N. Cholecystokinin accelerates the rate of habituation to a novel environment. Pharmacol. Biochem. Behav., in press.
3. Crawley, J.N. Divergent effects of cholecystokinin, bombesin, and lithium on rat exploratory behaviors. Brain Res. Bull., in press.
4. Crawley, J.N. and Schwaber, J.S. Nucleus tractus solitarius lesions block the behavioral actions of cholecystokinin. Peptides, in press.
5. Crawley, J.N. and Schwaber, J.S. Abolition of the behavioral effects of cholecystokinin following bilateral radiofrequency lesions of the parvocellular subdivision of the nucleus tractus solitarius. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 02178-01 NS
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Neuropharmacology of Anxiety		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Jacqueline N. Crawley, Ph.D., NSB, NIMH		
COOPERATING UNITS <i>(if any)</i> Laboratory of Bioorganic Chemistry, NIADDK, Section on Preclinical Studies, NSB, NIMH		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> The discovery of brain recognition sites specific for benzodiazepines opened the field for identifying specific brain mechanisms mediating anxiety. Animal models of anxiety are under development to address the questions of neuroanatomical sites specific for anxiety responses, receptor subtypes mediating anxiety responses, and known neurotransmitter systems activated or suppressed by anxiety-inducing drugs.		

Other Professional Personnel:

Steven M. Paul	Chief	NS NIMH
Philip T. Ninan	Clinical Associate	NS NIMH
Phil Skolnick	Pharmacologist	LBC NIADDK
John Glowa	Guest Worker	NS NIMH

Project Description:Objectives:

The recent discovery of benzodiazepine receptor antagonists led to intensive investigation of anxiogenic or anxiety-inducing properties of brain benzodiazepine receptor blockers. Our hypothesis rests on the postulated existence of an endogenous substance or substances which normally activate or/and inhibit the neuronal firing of cell groups containing benzodiazepine receptors. Pharmacological agents which block the receptor should then produce a response active but opposite to receptor agonists, just as isoproterenol stimulates and propranolol blocks β -adrenergic receptors, producing opposite cardiovascular effects. The objective of the project is the identification of anatomical and neurochemical sites of action of anxiety-producing and anxiety-reducing drugs.

Methods Employed:

Exploratory behavioral analysis of mice in a two-chambered light-dark environment; thirsty-lick conflict test in rats; behavioral rating of evoked anxiety-related behaviors in rhesus monkeys.

Major Findings:

The "active" benzodiazepine receptor antagonist, 3-carboethoxy- β -carboline (β -CCE) produced an extreme response in chair-adapted rhesus monkeys, ranging from mild to severe anxiety symptoms at 100 μ g/kg to 2 mg/kg i.v. A newly-designed behavioral rating system, using 22 parameters specific for anxiety in rhesus monkeys, was scored once a minute for 30 minutes after β -CCE administration. Behavioral responses correlated well with increases in blood pressure, heart rate, plasma cortisol, ACTH, and β -endorphin (Ninan). Effective pharmacological blockers of the β -CCE response were diazepam, clonidine (10 μ g/kg presynaptic dose), cyproheptadine, and morphine. Propranolol pretreatment produced only minimal blockade. These findings suggest that the α -2 noradrenergic receptors, serotonin receptors, and enkephalin receptors may be integral to the neural network which is activated by β -CCE administration.

A series of β -carbolines and other benzodiazepine receptor antagonists was similarly administered to mice in the light-dark exploratory model for anxiety. None of the antagonists tested had any intrinsic anxiogenic activity in this system, although all blocked the anxiolytic actions of diazepam. These results suggest that benzodiazepine receptor antagonists may be active anxiogenics in primates, less active in rats, and inactive in mice. Metabolism of β -CCE may be responsible for this difference. A ten-fold monkey/rat differential of in vivo half-life of β -CCE has been documented (Skolnick). These experiments emphasize the need for an anxiety-specific

primate model for the preclinical studies of benzodiazepine receptor antagonists in understanding the behavioral and somatic symptomology of anxiety.

Proposed Course of the Project:

The primate model of anxiety will be further developed to address major issues on the neuropharmacology of anxiety: involvements of opiate, GABA, and serotonin systems in the β -carboline response; 3) neuroanatomic site of the β -CCE induction of anxiety; 4) similarity to non-pharmacological, e.g. stress-induced anxiety; 5) blockade of the behavioral and somatic symptoms of stress-induced anxiety by neutral benzodiazepine receptor antagonists.

Significance to Biomedical Research and the Program of the Institute:

The primate model of anxiety induced by β -CCE appears to mimic the natural species-specific anxiety syndrome in rhesus monkeys. This model may provide new information on the pharmacology and anatomy of brain systems mediating panic attacks and anxiety neuroses.

Publications:

1. Crawley, J.N. and Moody, T.W. Anxiolytics block excessive grooming behavior induced by ACTH₁₋₂₄ and bombesin. Brain Res. Bull. 10: 399-401, 1983.
2. Crawley, J.N., Patel, J. and Marangos, P.J. Adenosine uptake inhibitors potentiate the sedative effects of adenosine. Neurosci. Lett. 36: 169-174, 1983.
3. Blumstein, L.K. and Crawley, J.N. Further characterization of a simple, automated exploratory model for the anxiolytic effects of benzodiazepines. Pharmacol. Biochem. Behav. 18: 37-40, 1983.
4. Skolnick, P., Paul, S., Crawley, J., Lewin, E., Lipka, A., Clody, D., Irmscher, K., Saiko, O. and Minck, K.O. Antagonism of the anxiolytic action of diazepam and chlordiazepoxide by the novel imidazopyridines, EMD 39593 and EMD 41717. Eur. J. Pharmacol. 88: 319-327, 1983.
5. Crawley, J.N., Blumstein, L.K. and Baldino, F. Anxiolytic-like properties of fominoben. Eur. J. Pharmacol., in press.
6. Crawley, J.N., Skolnick, P. and Paul, S.M. Absence of intrinsic antagonist actions of benzodiazepine antagonists on a mouse exploratory model of anxiety. Neuropharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 02179-01 NS

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Hamster Separation Model of Depression

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Jacqueline N. Crawley, Ph.D., NSB, NIMH

COOPERATING UNITS (if any)

Laboratory of Brain Evolution and Behavior, NIMH; Section on Preclinical Studies, NSB, NIMH; Office of Clinical Director, NIAAA

LAB/BRANCH

Clinical Neuroscience Branch

SECTION

Section on Molecular Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

A novel hamster separation model of depression is under development for analysis of central neurochemical changes during a behavioral state analogous to human depression. Separated hamsters showed increased body weight, decreased exploratory and social behaviors, and reduced serotonergic activity. The model may provide a robust rodent paradigm to investigate brain neurochemical and receptor changes during a behaviorally-induced "depressive state."

Other Professional Personnel:

Steven M. Paul	Chief	NSB NIMH
Richard Hauger	Staff Psychiatrist	NSB NIMH
Itzchak Angel	Guest Worker	NSB NIMH
Markku Linnoila	Clinical Director	NIAAA
Paul MacLean	Chief	LBEB NIMH

Project Description:Objectives:

Development and evaluation of a new rodent model for depression. Characterization of the changes in central receptor sensitivity and neurotransmitter turnover during the defined state of social separation in dwarf hamsters.

Methods Employed:

Breeding, pairing, and separating of dwarf hamsters; behavioral rating of social activities in pair-bonded Siberian dwarf hamsters; receptor binding assays; HPLC assays.

Major Findings:

The psychopharmacology of depression remains a complex issue, requiring better animal models for testing hypotheses of neurochemical abnormalities. The two animal models in current use are the Wisconsin monkey separation paradigm and the Seligman learned helplessness syndrome. Primate studies have the disadvantage of small sample size and limitations on studies of brain chemistry. Learned helplessness is an inescapable foot shock paradigm, which is primarily modeling the long term effects of stress. We are attempting to develop a better rodent model, which has both the advantages of large sample size and access for neurochemical assays, and closer conceptual and behavioral analogies to human depression. Phodopus sungorus is a rare species of Siberian dwarf hamster. It is reported to have a social system of male-female pair bonding, which is unusual among rodents. Anecdotal reports suggested that separation of the members of a male-female pair resulted in a behavioral syndrome with analogies to human depression.

A breeding colony of dwarf hamsters has now been established at NIMH in Poolesville (MacLean). Non-littermate males and females are being paired, separated, and repaired. Behavioral observations of the paired and separated states have been quantitated. Separated hamsters show increases in body weight, are less exploratory in a challenge novel environment, and less socially interactive with an unfamiliar animal of the opposite sex. Males show greater changes during separation than females. Preliminary HPLC assays of monoamines and metabolites from brain regions of paired and separated hamsters (Linnoila) found large decreases in serotonin turnover during the separated phase. The first drug trial using daily injections of imipramine during the separated period produced a reversal of approximately half of the affected parameters. This model shows promise as a novel rodent paradigm to investigate brain neurochemical and receptor changes during a behaviorally-induced "depressive" state.

Proposed Course of Project:

Characterization and validation of the new hamster depression model will be continued. Paired and separated hamsters will be analyzed for changes in brain β -adrenergic receptors, serotonin receptors, and serotonin reuptake sites (Hauger, Angel). Paired and separated hamsters will be analyzed for changes in brain catecholamine turnover (Linnoila). Drug trials with broad spectrum MAO inhibitors and more neurochemically-specific antidepressants will be performed, based on the receptor and metabolite findings.

Significance to Biomedical Research and the Program of the Institute:

Phodopus sungorus is a rapidly breeding, easily maintained species. Its usefulness as a model for human depression will be determined over the next two years. Such a model could be applied to the development of new classes of antidepressants. At the level of basic research, the model could provide a discrete population for testing current theories of neurochemical dysfunctions in depression.

Publications:

1. Crawley, J.N. Preliminary report of new rodent separation model of depression. Psychopharmacol. Bull., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER 701 MH 02180-01 NS	
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Electrophysiological Studies of Peptidergic Function in Mammalian Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Daniel Hommer, M.D., NSB, NIMH; Lana Skirboll, Ph.D., Pharmacologist, NSB, NIMH		
COOPERATING UNITS (if any) Biological Psychiatry Branch, NIMH; Laboratory of Bioorganic Chemistry, NIADDK		
LAB/BRANCH Clinical Neuroscience Branch		
SECTION Section on Molecular Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.) Using extracellular single unit recording techniques we have found that <u>cholecystokinin (CCK)</u> modulates the sensitivity of <u>dopamine (DA) autoreceptors</u> in the substantia nigra. Systemically administered CCK produces a DA autoreceptor supersensitivity. This supersensitivity may explain CCK's putative antipsychotic effect. We have also found that CCK excites neurons in the olfactory tubercle, and that only those neurons which are inhibited by the DA agonist apomorphine are excited by CCK. In addition to studying CCK/DA interaction, we have also been attempting to determine whether systemically administered CCK acts directly in the brain or through some peripheral mechanism. We have found that neither acute nor chronic vagotomy, nor even high cervical cord transection has any effect on CCK's ability to excite DA neurons. It appears that CCK's actions on the DA system are centrally mediated. In addition to examining peptide catecholamine interactions we have also used <u>single unit recording techniques</u> to study the effects of various <u>benzodiazepine</u> receptor agonists and antagonists on gamma aminobutyric acid (GABA)-sensitive neurons in the substantia nigra zona reticulata. We observed that the so-called "peripheral" benzodiazepine agonist Ro-5-4864 excited these neurons but this excitation was not reversed by the benzodiazepine antagonist Ro-15-1788. In contrast, 3-carboethoxy- β -carboline (BCCE) excites these neurons and was reversed by Ro-15-1788. BCCE also blocks the inhibitory effect of iontophoretic GABA on these neurons while benzodiazepines potentiate GABA.		

Other Professional Personnel:

Steven Paul, M.D.
 Phil Skolnick, Ph.D.
 Agu Pert, Ph.D.

Psychiatrist
 Pharmacologist
 Psychologist

NS NIMH
 LBC NIADDK
 BP NIMH

Project Description:Objectives:

Immunohistochemical studies have shown that there is a coexistence of dopamine (DA) and cholecystokinin (CCK) in a subpopulation of mesencephalic neurons which in the rat project primarily to limbic areas. Previously, we reported that these DA/CCK containing cells are excited by either systemically and iontophoretically administered CCK. This raises the question of the functional significance of DA/CCK coexistence (i.e., how does the peptide CCK and the classical catecholamine neurotransmitter, DA, interact to affect the activity of neurons).

Cholecystokinin/dopamine coexistence is only one example of the peptide/classical neurotransmitter localization within the same neuron. Given the growing number of reports of multiple neurotransmitters in the same neuron, it is conceivable that coexistence may be the rule rather than the exception. In any event, an understanding of the physiological interaction of DA and CCK at the level of the single neuron may shed light on the question of functional significance of coexistence in general.

The substantia nigra (SN) zona reticulata (ZR) is a region which contains a high concentration of GABA and benzodiazepine (BZ) receptors. Recently, several groups have shown that microinjections of GABA agonists into the SN but not into adjacent mesencephalic areas, blocks electrically-induced seizures in rats. These microinjections also block the limbic after discharge following kindled seizures. This suggests that the SN may be an important region involved in propagation of seizure activity as well as in modulation of limbic system function. Since BZ can block seizures and alter behavior presumably mediated by limbic regions, the SN ZR represents an ideal location in which to study BZ actions using electrophysiological techniques.

Methods Employed:

To investigate these question we used extracellular single unit recording techniques in male albino rats. Four ug/kg of CCK or its analogue ceruletide were administered systemically and their effect on the ability of the DA agonist, apomorphine, to inhibit medial A9 neurons was evaluated. These neurons are in a region which has a high proportion of cells containing both CCK and DA.

Major Findings:

Pretreatment with either CCK or ceruletide lead to significant "shift to the left" of the dose response curve for apomorphine induced inhibition of firing. The ED₅₀ of apomorphine for both CCK and ceruletide was 4 ug/kg while the ED₅₀ for the control group was 8 ug/kg. This suggests that systemically

administered CCK-like peptides can induce DA autorceptor supersensitivity. Iontophoretically applied CCK also is able to potentiate the inhibitory action of iontophoretically applied DA on medial A9 neurons. These results suggest that a possible mechanism of action for CCK's and ceruletide's reported behavioral and antipsychotic effects is their ability to induce an acute DA autoreceptor supersensitivity.

In addition to investigating CCK/DA interaction in presynaptic regions such as medial A9, we have also examined CCK/DA interaction in post-synaptic areas such as the olfactory tubercle. This limbic region possesses a dense innervation of DA/CCK terminals. We have found that olfactory tubercle neurons which are excited by systemically-administered CCK can be inhibited by the DA agonist apomorphine, while neurons which do not respond to CCK likewise are not inhibited by apomorphine. It appears that CCK acts only on these neurons which are also sensitive to DA.

Just as our recent work has begun to increase our understanding of the perplexing question peptide/catecholamine interaction, we have also been investigating the equally perplexing question of where in the nervous system CCK acts. Although we have found that systemically and iontophoretically administered CCK have similar effects on CCK/DA neurons, several behavioral effects of systemically administered CCK are abolished in vagotomized animals. This suggests that CCK's behavioral effects may be mediated via the peripheral nervous system. In an effort to determine whether the excitatory effects of CCK on DA neurons were mediated centrally or peripherally the effect of CCK on medial A9 cells was examined in four groups of rats: (1) acutely vagotomized animals; (2) chronically vagotomized rats; (3) rats with a unilateral lesion to the nucleus tractus solitarius, or (4) rats given a C-1 transection prior to recording. None of these procedures altered CCK's excitatory effect on medial A-9 cells, suggesting that the ability of CCK to enhance the activity of DA neurons is not mediated through a peripheral action but rather represents a central action of the peptide.

We have found that a β -carboline derivative (BCCE), a compound which binds to the BZ receptor and causes severe anxiety in primates, potentially excites neurons in the SN ZR. This excitation is reversed by the BZ antagonist RO-15-1788. In contrast, the "peripheral" BZ receptor ligand Ro-5-4864, although it also increased the activity of SN ZR neurons, could not be reversed by RO-15-1788. We have also found that several different BZs potentiate the action of iontophoretically applied GABA on SN ZR neurons, and that BCCE blocks GABA's inhibitory effect.

Projected Course:

We will extend the study of BZ effects to the SN lateralis (SNL). The SNL is a region which sends direct projections to the amygdala. Thus, the ability of intranigally administered GABA agonists to decrease amygdaloid after discharge and kindled seizures may be mediated through the SNL. In addition to electrophysiologically characterizing this region we also plan to investigate the anatomy of SNL-amygdaloid interconnection using retrograde tracing techniques. We will also look at the effects of limbic kindling on the sensitivity of SNL neurons to GABA, CCK, and DA.

We also plan to continue our investigation of DA/CCK interaction both in the SN and in limbic DA/CCK terminal regions. The ability of CCK to modulate the action of iontophoretically applied neurotransmitters will be examined. Proglumide, a putative CCK antagonist, will also be tested in both midbrain and limbic areas.

Significance to Biomedical Research and the Program of the Institute:

There already have been two independent studies reporting that CCK and CCK-like peptides possess an antipsychotic effect. Our studies of DA/CCK interactions provide a possible model for how this action of CCK may be mediated. We are currently completing a study of the behavioral effects of ceruletide, a CCK-like peptide, in schizophrenic patients and in normal volunteers. Further clinical research with CCK-like peptides on eating disorders such as bulimia is now being planned. On a more basic level the study of the interaction between CCK and DA may provide a prototype for interactions between the peptide/classical neurotransmitter coexisting system.

Electrophysiological studies of BZ and GABA compliment ongoing biochemical and behavior studies in these areas. The SN appears to be an important region in the modulation of seizures. Electrophysiological techniques are particularly well suited to further our understanding of how the SN performs this function.

Publications:

1. Hommer, D.W. and Pert, A. The actions of opiates in the rat substantia nigra: an electrophysiological analysis. Peptides, in press.
2. Hommer, D.W. and Skirboll, L. Cholecystokinin-like peptides potentiate apomorphine-induced inhibition neurons. Eur. J. Pharmacol., in press.
3. Weissman, B.A., Cott, J., Hommer, D.W., Quirion, R., Paul, S.M. and Skolnick, P. Pharmacological, electrophysiological, and neurochemical actions of RO-5-4864 (4'chlorodiazepam). In Biggio, G. (Ed.): Benzodiazepine Recognition Site Ligands: Biochemistry and Pharmacology. New York, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00851-19 LBEB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Mechanisms of Display Behavior in Squirrel Monkey (<i>Saimiri sciureus</i>).		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Paul D. MacLean, M.D., Chief, Laboratory of Brain Evolution and Behavior, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Brain Evolution and Behavior		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 0.3	OTHER: 1.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In this long-term investigation we are utilizing the naturally occurring mirror display of one variety of squirrel monkey as a means of identifying genetically constituted forms of behavior used in social communication. In previous work on this project it was shown that the integrated performance of the display depends on neural systems that converge in the medial pallidal segment of the striatal complex, a phylogenetically ancient formation of the forebrain derived from reptiles. The medial pallidal segment projects to the dorsal thalamus and to parts of the thalamic and midbrain tegmentum. The present experiments represent a continuing effort to identify core gray matter and tegmental structures in the caudal thalamus, midbrain, and isthmus region involved in the three main aspects of the display--namely, vocalization, thigh-spread, and genital tumescence. The findings indicate that the core gray matter and the immediately adjacent tegmentum in the caudal thalamus, as well as in the caudal part of the midbrain adjoining the isthmus region, is particularly implicated in the vocal aspects of the display. In regard to special nuclei associated with the isthmus region, it has been shown that the serotonin-containing cells of the superior central nucleus of Bechterew, as well as the ascending system from the dorsal tegmental nucleus of Gudden, are not essential for the complete and consistent performance of the display. </p>		
(779)		

Others Engaged in Project:

Thalia K. Bussard	Biologist	LBEB NIMH
Robert E. Gelhard	Biologist	LBEB NIMH
Carroll R. Harbaugh	Biol. Lab Tech.	LBEB NIMH

Project Description:

Objectives: The goal of this long-term study is to identify brain structures involved in species typical, prosematic communication. Prosematic, meaning rudimentary signaling, applies to any nonverbal signal--vocal, bodily, or chemical--used in communication. Basically, terrestrial vertebrates communicate by means of four main kinds of behavior typified by displays referred to as signature, challenge, courtship, and submissive displays. For investigation of the underlying brain mechanisms in a primate we have used a variety of squirrel monkeys (gothic-type) that performs a mirror display incorporating features of the signature, challenge, and courtship displays. It was shown in earlier work in this project that the organization of the somatic and autonomic features of the display depend on neural systems that converge in the medial pallidal part of the striatal complex--an evolutionary ancient formation of the forebrain reflecting an inheritance from reptiles.

In a continuation of the experiments reported last year, the present project is concerned with identifying which structures of the core gray matter and tegmentum of the caudal thalamus, midbrain, and isthmus are implicated in different manifestations of the display. The isthmus region is of special interest because it is not only a phylogenetically ancient part of the brainstem involved in the integration of somatovisceral functions, but is also the location of monoamine-containing cell groups and particular nuclei that project to the forebrain.

Methods Employed: The displays of squirrel monkeys are species-typical, being genetically determined. For this project we use mature males of the gothic-type variety (so-called because the ocular patch has the shape of a gothic arch) that unlike the roman-type (ocular patch with round arch) consistently display to their reflections in a mirror. The two varieties show karyotypic differences. Testing of the mirror display is performed twice a day and the results tabulated with respect to the five main components of the display. The occurrence of full genital tumescence, vocalization, and thigh-spreading ranks as a trump display. After achieving criterion performance (trump displays in 80% of 30 or more trials), a monkey undergoes surgery for electrocoagulation of targeted structures. After an animal attains plateau performance in a series of three sets of 30 trials, it is sacrificed and serial brain sections are prepared for histological examination and volumetric measurements of the reconstructed lesions.

Major Findings:

Isthmus Region. Thus far the outcome of experiments involving lesions of isthmus-related structures has been negative. In one animal (Q-5) there was remarkably complete spherical destruction of the superior central nucleus of Bechterew that contains serotonin cells innervating the hippocampus and other

structures of the forebrain. The consistent mirror display performance of this animal was unaffected. Significantly, also, the lesion resulted in extensive retrograde degeneration of the deep tegmental nucleus of Gudden as a consequence of interrupting its projections. In another animal (R-5) a small electro-coagulation involving the caudal part of the deep tegmental nucleus resulted in no change in the performance of the mirror display. In a third animal (V-5), still undergoing tests, an attempt was made to produce a sufficiently large lesion to encompass one or more of the neighboring isthmus nuclei near the midline--namely, the dorsal raphe nucleus containing serotonin cells; the deep tegmental nucleus of Gudden; and the locus caeruleus with norepinephrine-containing cells. This animal has shown no change in its consistent performance of mirror displays.

Caudal thalamic and midbrain tegmentum. Further experiments attempting to differentiate the role of thalamic and midbrain tegmental structures in the performance of the display have revealed the following interesting findings on vocalization: In one animal (S-5) with a remarkably symmetrical, discrete lesion destroying the periventricular gray matter at the level of the posterior hypothalamus, together with the origin of the medial longitudinal fasciculus rostral to the interstitial nucleus of Cajal, there was a selective elimination of the vocal component of the mirror display. The same was true of another monkey (T-5) in which there was a bilaterally symmetrical lesion of the lateral central gray and adjacent tegmentum involving part of the central tegmental tract at the caudal level of the inferior colliculus. Otherwise these animals achieved a perfect score in the mirror display test. In a third animal (U-5), a lesion was aimed at the fusion of the ansa lenticularis and thalamic fasciculus near the caudal level of the thalamic tegmentum. This monkey was hypothermic for five weeks and regulated its daytime temperature within normal limits by moving into and out of the warmth of a heat lamp. At about the same time it began to perform occasional mirror displays, but without vocalization. Such fragmented displays were consistently performed after the seventh postoperative week; during the course of four sets of 30 trials there have been only occasional weak vocalizations.

Nigrotectal projections. In a first attempt to investigate the role of the nigrotectal projections in the display, bilateral lesions were placed in the midbrain of one animal (W-5) in a plane calculated to interrupt most of the fibers in question. Following surgery, this monkey developed a Wilson-like athetosis in its upper extremities, as well as a resting tremor suggestive of that seen in Parkinson's disease. When it lifted a piece of food to the mouth, however, the tremor became greatly exaggerated, being similar to a cerebellar intention tremor. Despite this dystonic condition (still manifest after three months), this monkey quickly regained its preoperative level of performance in the mirror display (see also accompanying report No. Z01 MH 787-04 LBEB regarding its isolation call).

Additional findings. In one monkey (R-5) used in the study of the isolation call (see accompanying report) it was found that aspiration of a strip of limbic cortex extending from above the knee of the corpus callosum down to, and including, the posterior part of the gyrus rectus, together with an extensive area of the medial frontal neocortex, resulted in a

fragmentation of the mirror display throughout the following eight months of testing. Although this animal continued to display full erection in a 100% of the trials, there was a statistically significant decline in the vocal and thigh-spread components of the display. This outcome is to be weighed in the light of full preservation of the display in a monkey (S-4) reported in earlier work on this project that had been subjected to a bilateral frontal lobectomy back to the level of the knee of the corpus callosum. In this animal there was preservation of the entire strip of limbic cortex in question except the pregenual cingulate cortex.

Significance to Biomedical Research and the Program of the Institute:

In its evolution, the primate forebrain has expanded to human size while retaining the anatomical and biochemical features of three basic formations that reflect an ancestral relationship to reptiles, early mammals, and late mammals. The mammalian striatal complex, consisting of the corpus striatum and olfactostriatum and related structures, represents an elaboration of the paleostriatum and olfactostriatum of the reptilian and avian forebrain. Based on comparative findings in this and related projects, it has become evident that neural systems converging within the striatal complex play a basic role in the integration of displays used by reptiles and mammals in social communication. The findings of the current project contribute to an identification of tegmental structures in the thalamus and midbrain that are specifically implicated in the vocal component of squirrel monkey displays. In addition, it has been shown that partial elimination of certain monoaminergic cell groups and other nuclear structures of the isthmus that project to the forebrain results in no change in the display performance scores of the squirrel monkey. In evaluating the significance of experimental findings of this kind in regard to functions of the human brain, it is to be emphasized that human beings rely in large measure on the same forms of behavior as other mammals for social communication.

Proposed Course: To be continued.

Publications:

MacLean, P.D.: Evolution of the psychencephalon. Zygon 17: 187-211, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00787-04 LBEB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Mechanisms of Isolation Call in Squirrel Monkey (<i>Saimiri sciureus</i>).		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Paul D. MacLean, M.D., Chief, Laboratory of Brain Evolution and Behavior, NIMH		
COOPERATING UNITS (if any) Laboratory of Developmental Neurobiology, NICHD		
LAB/BRANCH Laboratory of Brain Evolution and Behavior		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.3	OTHER: 0.9
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This continuing investigation is concerned with identifying cerebral mechanisms accounting for the structure and production of the isolation call. This vocalization, which appears to be common to all mammals, is perhaps the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. In a comparative approach to this problem we are utilizing squirrel monkeys (<i>Saimiri sciureus</i>) which have a typical isolation call that lends itself to experimental analysis. This year's findings have helped to delineate further the core gray matter and adjacent tegmental structures at the junction of the thalamus and midbrain (see Z01 MH 00787-01 LBEB) that appear to be essential for the patterning and production of the isolation call. This region is known to receive projections from the medial frontal limbic cortex. Our experiments thus far indicate that ablation of the entire strip of limbic cortex extending from the anterior cingulate down to, and including, the posterior part of the gyrus rectus, permanently interferes with the spontaneous production of the isolation call. Incomplete ablation of this strip or aspiration of the medial frontal neocortex at the level of the genu results in only a temporary elimination of spontaneous calls. Finally, in regard to vocal effector mechanisms, it is notable that bilateral destruction of the lateral central gray and adjoining tegmental structures at the caudal level of the inferior colliculus eliminated all the recognized calls of squirrel monkeys except cackles. The region in question is believed to encompass an effector pathway leading to Nucleus ambiguus. </p>		
(783)		

Co-investigator: John D. Newman, Research Physiologist, LDN, NICHD, NIH

Others Engaged in Project:

Thalia K. Bussard	Biologist	LBEB	NIMH
Robert E. Gelhard	Biologist	LBEB	NIMH
Carroll R. Harbaugh	Biol. Lab. Tech.	LBEB	NIMH

Project Description:

Objectives: The purpose of this investigation is to identify the neural network of cerebral structures involved in the patterning and production of the isolation call, perhaps the most primitive and basic mammalian vocalization. In the evolution from reptiles to mammals, three key developments were (i) nursing in conjunction with maternal care, (ii) audiovocal communication for maintaining maternal-offspring contact, and (iii) playful behavior. Significantly, it now appears that in the brain's evolution there has occurred a representation of this behavioral triad in the cingulate gyrus, a recent evolutionary development within the limbic system for which there is no counterpart in the reptilian brain. Inclusive of the thalamocingulate division, the limbic system represents an inheritance from early mammals. From the standpoint of understanding human evolution, there could hardly be anything more important than reconstructing the steps by which the three forms of behavior in question have developed. In a comparative approach to this problem, we are using squirrel monkeys for inquiring into the part played by the cingulate gyrus in the isolation call. In earlier work on this project (Z01 MH 787-03 LBEB) it was found that the core gray matter and adjacent tegmental structures at the junction of the thalamus and midbrain are implicated in the patterning and production of the isolation call. These structures are known to receive a projection from the rostral cingulate cortex. The present report deals with the effects of rostral limbic and medial frontal neocortical ablations on the isolation call. In addition, supplementary findings are described in regard to midbrain mechanisms of vocalization.

Methods: Subjects are mature male and female squirrel monkeys of either the gothic or roman types (see accompanying report, Z01 MH 851-19 LBEB, regarding these varieties). In addition to certain components of their displays, the two varieties produce slightly varied isolation calls that show up as distinct differences in the sound spectrogram. The two varieties of monkeys also have distinctive karyotypic differences. For inducing isolation calls, monkeys are placed in a sound attenuating chamber. Criterion performance is the production of 20 isolation calls in 15 minutes. If an animal fails to reach criterion, testing is continued for 15 minutes while it listens to conspecific calls. Recordings are made with a standard Uher microphone, and sound spectrograms are obtained with a VII model 700 sound spectrogram. After a monkey reaches criterion, standard operative procedures are used for aspirating cortical areas or for producing lesions of subcortical structures by electrocoagulation.

Major Findings: As to be described, the results of cortical operations indicate that ablation of a strip of rostral limbic cortex eliminates the spontaneous production of isolation calls, while supplementary experiments on the brainstem have given additional information about structures involved in the patterning and production of isolation calls.

Cortical ablations. Earlier studies by German workers had indicated that ablations of the cortical face area, the major part of the supplementary area, and the anterior cingulate gyrus (including the pregenual cortex) had no effect on various spontaneous vocalizations of squirrel monkeys, including the isolation call. Ablation, however, of the "anterior" supplementary area at the level of the genu of the corpus callosum is said to have resulted in a reduction of spontaneous calls, with the most marked effect on the isolation call. It is to be emphasized, however, that these workers did not extend their ablations into the infragenual limbic cortex and also did not follow their animals for longer than one month. In one female gothic-type monkey (C-1), we removed the "anterior" supplementary area. Although this animal did not produce spontaneous isolation calls for the first three weeks, she regained both the preoperative structures and rate of production of the calls by nine weeks. At the time of last year's progress report the findings on two critical animals with medial frontal ablations were not complete. In the case of one male gothic animal (R-5) with aspiration of a rostral strip of limbic cortex including the anterior supracallosal cingulate cortex, the pregenual cingulate cortex, and the cortex of the subcallosal gyrus and posterior part of the gyrus rectus, there was a failure to produce spontaneous isolation calls throughout eight months of observation. Towards the end of that period, the monkey made a few isolation calls in response to tape-recorded calls. In the case of the other animal (No. 914, roman-type female) in which there was sparing of the anterior cingulate cortex above the corpus callosum and part of the posterior part of the gyrus rectus, there was a return to the level of the preoperative production of the calls within a week's time. In both monkeys the ablations had included an extensive part of the prelimbic medial frontal neocortex.

Lesions of the brainstem. In two animals described in a preceding report (Z01 MH 00787-01 LBEB), lesions of the core gray matter and adjacent tegmentum at the junction of the thalamus and midbrain resulted in an alteration of the structure of the isolation call. The tegmental lesions common to both animals were an almost a complete destruction of the interstitial nucleus of Cajal and the nucleus of Darkschewitsch. Evidence that one or both of these nuclei may be implicated in the call is derived from the findings in monkey S-5 in which there was a remarkably symmetrical small lesion of the periventricular gray matter and origin of the medial longitudinal fasciculus at the level of the posterior hypothalamus that impinged only on the rostral part of the interstitial nucleus. The monkey in this case failed to vocalize in the mirror display test (see accompanying report Z01 MH 851-19 LBEB), and though failing to reach criterion in the production of isolation calls, the structure of the call was normal.

The findings in another animal (T-5) provided dramatic evidence supporting the inference of previous workers that a fiber system issuing from the central gray at the caudal level of the inferior colliculus may be the major effector pathway for vocalization. The efferent nerves in question have been described as passing laterally through the parabrachial region and then descending towards Nucleus ambiguus. In the monkey under consideration, the lesion involved the lateral central gray and adjacent tegmentum at the caudal level of the inferior colliculus. Subsequent to surgery, the monkey was so avocal under all conditions (including being caught for weighing) that his keepers referred to him as

"Silent Sam." The only vocalizations heard during the eight-month follow-up were a few cackles when the monkey was shown a fur-covered dummy, as well as a few short atypical sounds emitted during some mirror display tests. Since the lesion in this case interrupted part of the central tegmental tract, it is possible that this phylogenetically ancient fiber system also forms part of the effector mechanism. As noted in a preceding project report (Z01 MH 00787-01 LBEB), lesions involving this tract and lateral central gray at other levels of the midbrain result in an almost complete failure to produce isolation calls, whereas other vocalizations are intact.

Lesions with negative effects. In one animal (Q-5) with a lesion of the superior central nucleus of Bechterev (which has a population of serotonin-containing cells that innervate the hippocampus and other parts of the forebrain), there was no effect on the animal's ability to produce isolation calls. It is also noteworthy that in this animal there were retrograde changes in the deep tegmental nucleus of Gudden, resulting from an interruption of its projections by the lesion. In another animal (R-5) with a small electrocoagulation that partially destroyed the caudal part of the deep tegmental nucleus of Gudden, there was no effect on the isolation call. In a third animal (W-5) still undergoing testing, bilateral lesions were placed in the ventral midbrain, with a resulting Wilson-like athetosis and a tremor of the upper extremities partially mimicking Parkinsonian tremor while the animal was at rest, but resembling a cerebellar intention tremor when the monkey lifted food to its mouth. In this animal there was no effect on the isolation calls.

Ancillary Findings

As described in last year's report (Z01 MH 00781-03 LBEB), the administration of morphine results in an elimination of isolation calls, whereas its antagonist naloxone reinstates the calls. Naloxone given alone does not have the expected effect of increasing the rate of production of the calls. Both for experimental purposes and for gaining further knowledge of the underlying mechanisms, it would be desirable to identify substances that would increase the rate of production of isolation calls. Three suggested such agents--gonadotrophin-releasing hormone (monkeys U-5, V-5, W-5), oxytocin (395K; T-5; 930; Z-4), and amphetamine (P-085, Q-5)--proved to be ineffective in this respect.

Significance to Biomedical Research and the Program of the Institute:

As noted under objectives, research by ourselves and others indicates that there is a representation in the cingulate gyrus of three forms of behavior that distinguish the evolutionary transition from reptiles to mammals. The cingulate gyrus is phylogenetically the newest part of the limbic system of mammals. Significantly, it has no counterpart in the reptilian brain. It is postulated that the isolation call, which is the focus of the present study, may be the most primitive and basic mammalian vocalization, serving originally to maintain maternal-offspring contact. The attempt to identify cerebral mechanisms accounting for its structure and production may eventually contribute to an understanding of the evolutionary link-up between emotional vocalization and propositional speech in human beings. Our original findings indicated that tegmental structures and core gray matter at the junction of the thalamus and

midbrain are essential for the patterning of the call. The core gray matter in question is continuous with the central gray of the midbrain that has long been recognized to be basically involved in vocalization. One finding in the present study indicates that, in agreement with observations of a group of workers elsewhere, a pathway issuing from the caudal central gray and tegmentum may be a major effector pathway for several forms of vocalization, including the isolation call. In addition, our current findings provide evidence that a strip of rostral limbic cortex is effective in regulating the production of the isolation call. In view of the distressful nature of the isolation call, it is of special interest that opiate receptors have been found in high concentration in both the central gray matter and in the cingulate gyrus, and that the administration of morphine eliminates both maternal behavior and the isolation call. Such considerations illustrate how the findings in the present study may eventually prove relevant to psychological disorders of concern to mental health, as, for example, anxiety of separation, the "failure-to-thrive" syndrome, prolonged grief reactions, depression, and opiate addiction.

Proposed Course: To be continued.

Publications:

MacLean, P.D.: The Co-evolution of the Brain and Family. In Brady, E.P., Waldron, E., and Slater, J. (Eds.): Anthroquest, Pasadena, The L.S.B. Leakey Foundation News, No. 24, Winter/T982, pp. 1 and 14-15

Newman, J.D.: The Infant Cry of Primates: An Evolutionary Perspective. In Lester, B.M., and Boukydis, C.F.Z. (Eds.): Infant Crying: Theoretical and Research Perspectives. New York, Plenum Publishing Corp. (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00793-02 LBEB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Brain Iron and Neuroendocrine Regulation		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Joanna M. Hill, Visiting Fellow, Laboratory of Brain Evolution and Behavior, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Brain Evolution and Behavior		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 0.6	OTHER: 0.4
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> We previously demonstrated that the iron concentration in the <u>globus pallidus</u> and <u>substantia nigra</u> (measured with <u>spectrophotometry</u>) <u>fluctuates</u> during the <u>estrous cycle</u> and <u>pregnancy</u>. Peak concentrations occur at the same time as the sex hormones <u>estrogen</u>, <u>progesterone</u>, <u>prolactin</u>, and <u>luteinizing hormone-releasing hormone</u> are at highest serum levels. In addition, we have shown that the introduction of an iron chelator, <u>desferal mesylate</u>, into the third ventricle interrupts estrous cyclicity. These studies link <u>brain iron</u> to <u>neuroendocrine regulatory mechanisms</u>. </p> <p> The present three-part study is designed to learn: (1) whether the fluctuations in iron during the estrous cycle occur in all or in only select iron-concentrating brain sites as determined by the combined use of the <u>diamino benzidine intensified Perls' reaction</u> for ferric iron and <u>densitometry</u>; (2) whether several <u>sequential pregnancies</u> deplete brain iron stores as determined with both <u>spectrophotometric</u> and <u>histochemical</u> methods; and, (3) if induced changes in <u>caudate-putamen neurotransmitter metabolism</u> affect the accumulation of iron in the <u>globus pallidus</u> and <u>substantia nigra</u>, which receive <u>striatal input</u>. The results suggest that the accumulation of iron in specific brain areas is due to the association of iron with <u>neurotransmitter metabolism</u>. </p>		

Project Description:

Objectives: The present project is part of an investigation on the physiological role of iron found in high concentration in the striatal structures of the basal forebrain. In previous studies, using chemical methods, we demonstrated in the rat that the iron content of the pallidal component of the striatal complex and the substantia nigra doubles during the proestrus phase of the estrous cycle and also rises significantly during the first third of pregnancy. The rise in iron occurs in conjunction with an elevation of the hormones estrogen, progesterone, prolactin, and luteinizing hormone-releasing hormone. Histochemical techniques now in use have made it possible to detect small amounts of iron in tissue and, with densitometric analysis, to measure its relative concentration (see Z01 MH 00871-07 LBEB). These methods afford measurements of iron in brain areas too small to be measured chemically.

The present report gives the findings to date of three studies designed to show: (1) whether the proestrus surge in brain iron occurs in all, or in only select, iron-concentrating brain areas; (2) if multiple pregnancies affect brain iron reserves; and (3) if induced changes in the neurotransmitter metabolism of the caudate-putamen will affect the accumulation of iron in the two main structures receiving its projections--namely, the globus pallidus and substantia nigra.

Methods Employed:

Study 1. After determining estrous cyclicity by the vaginal smear technique, four groups of five, 27-week-old nulliparous female rats are sacrificed on each of four days of the estrous cycle (proestrus, estrus, metestrus, and diestrus). The brains are cut on a freezing microtome at 50 μ m and stained for iron with the diaminobenzidine intensification of the Perls' reaction for ferric iron. The intensity of the stain in iron-concentrating areas is measured with a Zeiss microscope equipped with a densitometer.

Study 2. Samples of globus pallidus, substantia nigra, and cortex are obtained from 279 μ m thick slabs of rat brain by micropunch. Iron levels in the above brain areas are determined by spectrophotometry, as are also the iron levels in the serum and liver. Two groups of six animals are examined: (1) "breeders" 38-42 weeks of age that have produced 4 to 7 litters, and (2) control females of the same age which have never bred. In addition, iron histochemistry and densitometry are performed on six, 27-week-old females having had three litters prior to sacrifice. The brain iron concentration of these animals is compared with that of the females in Study 1.

Study 3. Unilateral electrolytic coagulation within the caudate-putamen, or injection with either colchicine or gamma-vinyl gamma-aminobutyric acid (gamma-vinyl GABA), is used to disrupt neurotransmitter input to the iron-concentrating globus pallidus and substantia nigra. After survival times of 1 to 3 days, the animals are sacrificed, and the brains stained for iron. The unoperated side of the brain serves as a control.

Major Findings:

Studies 1 and 2. To date, the findings indicate that 27-week-old females accumulate sufficient brain iron to provide a measureable histochemical stain, while being young enough for the fluctuations in hormone levels to occur.

Study 3. To date, it has been found that two days after respective treatment with colchicine or gamma-vinyl GABA, the iron in pars reticulata of the substantia nigra is reduced by 8-48% and 22-64%. In addition, there is a 2-29% reduction in pallidal iron on the side of the brain injected with colchicine. This reduction is more evident in the anterior parts of the globus pallidus. In gamma-vinyl GABA injected brains a 5-20% increase of iron was found in the globus pallidus on the injected side. The oligodendrocytes appear to be more darkly stained and the neuropil paler than on the control side. In animals sacrificed two days after unilateral electrocoagulation of the caudate-putamen there is no measurable change in the amount of iron in the globus pallidus or substantia nigra.

Significance to Biomedical Research and the Program of the Institute: Although occurring in small quantities, iron enzymes play an important role in brain metabolism. Iron is a requirement for oxidative metabolism, several monoamine synthetic and degradative enzymes, and plays a role in the maintenance of monoamine oxidase levels. Iron is also involved in serotonin-binding, glutamate-binding, and dopamine-receptor function. Iron deficiency is a common nutritional disorder, especially in women of child bearing age. In studies on rats, the behavioral symptoms occurring in iron deficiency have been linked to changes in the metabolism of the monoamine systems. The present study will provide important information concerning the extent of the change in brain iron mobilization and its availability during hormonal fluctuations. It is also designed to reveal the effects of frequent pregnancies on reserves of brain iron. The results of Study 3 support the hypothesis that the accumulation of iron in specific areas is due to the association of iron with neurotransmitter metabolism. Inhibition of axoplasmic transport in the caudate-putamen by colchicine reduced pallidal and nigral iron, while blocking GABA degradation with gamma-vinyl GABA resulted in an increase of pallidal glial iron and a reduction in total iron in the substantia nigra.

Proposed Course: To be continued.

Publications:

Hill, J.M.: Brain Iron: Sex Difference and Changes During the Estrous Cycle and Pregnancy. In Saltman, P., and Hagenauer, J. (Eds.): The Biochemistry and Physiology of Iron. Proceedings of the Fifth International Conference on Proteins of Iron Storage and Transport. New York, Elsevier North Holland, 1982, pp. 599-601.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00871-07 LBEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

A Histochemical Study on the Location of Brain Iron

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Joanna M. Hill, Visiting Fellow, Laboratory of Brain Evolution and Behavior, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL YEARS:

1.0

PROFESSIONAL:

0.6

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The diamino benzidine intensification of the Perls' reaction for ferric iron has been used for both the light and electron microscopic localization of iron in the brain. Microscopic examination has revealed the the consistent presence of iron in fifty-one structures in the rat brain. The relative concentration of iron at these sites has been determined by densitometry. In most brain areas iron is found in oligodendrocytes. Several select areas, however, concentrate iron in neurones. The pattern of iron distribution is similar to that of gamma-aminobutyric acid (GABA) and also overlaps to a considerable extent with that of enkephalins and luteinizing hormone-releasing hormone. The possibility that iron accumulation is related to the involvement of iron in the metabolism of neurotransmitter systems is further supported by the results of experiments described in an accompanying report (Z01 MH 00793-02 LBEB).

Electron microscopic localization of iron has shown that in oligodendrocytes, iron is present throughout the cytoplasm, in association with mitochondria and the Golgi apparatus, and in the inner and outer myelin sheath. Neuronal iron is localized in membrane bound structures (possibly lysosomes), in association with the Golgi apparatus, and in the dendritic cytoplasm. This suggests that brain iron may play an important role in the synthetic and secretory functions in iron-containing cells.

Project Description:

Objectives: In connection with our studies on the anatomy and functions of the striatal complex, we have investigated the localization of iron in the brain. This report summarizes the final outcome of the light microscopic localization of brain iron and the preliminary findings with electron microscopy.

It is known that iron is essential for brain function and that iron deficiency results in behavioral disorders (see accompanying report No. 793-02). The aim of this study is to gain a better understanding of the role of iron in the brain, particularly in structures such as the pallidum and substantia nigra where it is highly concentrated. By mapping the distribution and relative concentration of iron in various structures it will be possible to compare its pattern of distribution with that of several known biologically active substances (e.g., GABA, enkephalins, and LHRH). The determination of its subcellular location by means of electron microscopy is an essential part of the study.

Methods Employed: The di amino benzidine intensification of the Perls' reaction for ferric iron is used for both the light and electron microscopic localization of iron in the brain (see preceding report Z01 MH 00871-06 LBEB). The brains of five, 35-week-old female rats, all in the proestrus stage of the estrous cycle, were systematically examined, and quantitative measures of the intensity of stain were obtained by densitometry.

Major Findings: Microscopic examination and densitometric readings revealed that 51 structures in the rat brain showed a consistent presence of iron. The loci and concentration are listed in Table 1. In most brain areas iron occurs in oligodendrocytes and throughout the neuropil. In several areas, however, iron occurs in association with neurons. In the circumventricular areas it is present either in ependymal cells or interstitial spaces. Tanycytes in the median eminence and organum vasculosum show a particularly high concentration (see Table 1).

Electron microscopic examination has revealed that iron in oligodendrocytes occurs in association with mitochondria; the Golgi apparatus; and throughout the cytoplasm, continuing into the inner and outer myelin sheaths formed by the oligodendrocytes. Neuronal iron is present in the Golgi apparatus and cytoplasm of dendrites, as well as within lysosome-like structures.

Significance to Biomedical Research and the Program of the Institute: On the basis of what is known about the cerebral localization of gamma-aminobutyric acid (GABA), enkephalins, and luteinizing hormone-releasing hormone (LHRH), a study of Table 1 suggests that the distribution of iron closely parallels that of GABA and also overlaps to some extent with enkephalins and LHRH. That the accumulation of iron in specific areas is due to its association with neurotransmitter metabolism is supported by the following evidence detailed in an accompanying report (Z01 MH 00793-02 LBEB). Inhibition of axoplasmic transport by colchicine in the caudate-putamen resulted in a reduction of pallidal and nigral iron, whereas blocking GABA degradation with gamma-vinyl GABA increased pallidal glial iron and reduced total iron in the substantia nigra. The association of

Table 1. Distribution of Iron in the Rat Brain

The intensity of stain values, obtained by densitometry, are the means + standard errors of the percentage of light absorbed. Abbreviations under Cellular localization: E, iron in ependymal cells; I, interstitial iron; G, iron in glia and fibrous network of the neuropil; N, iron in or upon the perikarya and neuronal processes of nerve cells.

<u>Area</u>	<u>% of light absorbed</u>	<u>Cellular localization</u>
Subfornical organ	57.5+3.26	E, I
Islands of Calleja of the olfactory tubercle	52.9+5.68	N
Organum vasculosum of the lamina terminalis	46.3+2.93	E
Median eminence	41.9+2.41	E
Area postrema	39.5+5.70	E, I
Ventral pallidum	35.6+4.96	G
Globus pallidus	35.6+3.44	G
Interpeduncular nucleus	35.6+2.21	G
Dentate and Interpositus nuclei	34.5+1.80	G
Substantia nigra pars reticulata	34.4+1.89	G
Superior olive	30.9+2.27	G
Facial nucleus	26.0+3.19	G
Olfactory bulb glomerular and granular layers	25.1+1.83	G
Dorsal tegmental nucleus	24.9+2.69	G
Inferior olive	24.1+1.68	G
Olfactory tubercle plexiform layer	22.9+1.98	G
Entopeduncular nucleus	22.5+1.42	G
Hypoglossal nucleus	22.4+1.48	G
Inferior colliculus	22.4+3.35	G
Lateral lemniscus	22.4+2.41	G
Fastigial nucleus	21.5+0.98	G
Lateral habenula	21.2+0.41	G
Ventral thalamic nuclei	21.0+1.89	G
Cochlear nucleus	20.5+1.78	G
Caudate putamen fiber bundles	19.2+2.52	G
Cerebellar granular layer	18.3+1.26	G
Mammillary nuclei	17.0+1.46	G
Hippocampus CA 3	16.7+1.88	G
Superior and medial vestibular nuclei	15.5+2.22	G
Superior colliculus	15.4+1.73	G
Paraventricular nucleus	15.1+4.14	G, N
Spinal trigeminal nucleus	14.4+1.64	G
Parvocellular reticular nucleus	14.3+1.64	G
Anterior pretectal area	14.2+0.81	G
Raphe nuclei	13.6+2.11	G
Lateral septum	13.4+1.67	N
Mediodorsal thalamus	12.9+1.94	G
Central gray	12.8+1.05	G
Lateral geniculate nucleus	12.7+0.61	G
Medial geniculate nucleus	12.3+1.17	G

<u>Area</u>	<u>% of light absorbed</u>	<u>Cellular localization</u>
Lateral hypothalamic area	11.7±0.58	G
Dentate gyrus	11.6±1.39	N
Bed nucleus of the stria terminalis	11.2±2.27	N
Suprachiasmatic nucleus	11.1±3.32	N
Central amygdala	9.9±1.78	N
Cingulate cortex	9.2±0.07	G,N
Deep mesencephalic nucleus	9.2±1.02	G
Lateral vestibular nucleus	9.2±0.74	N
Supraoptic nucleus	8.6±1.27	G,N
Cerebral cortex	8.1±0.53	G
Oculomotor nucleus	7.0±1.44	N

iron with the Golgi apparatus in both neurones and oligodendrocytes further suggests that iron plays an important role in the synthetic and secretory functions of those cells which accumulate it.

Brain iron increases with age, and there is a clinical report that the globus pallidus accumulates iron to the same extent as the liver. Iron acts as a catalyst in lipid peroxidation and, thus, is potentially harmful where it occurs in high concentration. Such considerations raise the question of the possible deleterious role of iron in neurological disorders such as Parkinson's disease, Huntington's chorea, Alzheimer's dementia, schizophrenia, and manic-depressive disorders.

Proposed Course: To be continued.

Publications:

Switzer, R., Hill, J. and Heimer, L.: The globus pallidus and its rostroventral extension into the olfactory tubercle of the rat: A cyto- and chemoarchitectural study. Neuroscience 7: 1891-1904, 1982.

Hill, J.M., and Switzer, R.C.: The regional distribution and cellular localization of iron in the rat brain. Neuroscience (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00847-03 LBEB
PERIOD COVERED October 1, 1982 to August 20, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of the Neocortex in Coping with Complexity		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) James L. Hill Visiting Associate URBS, LBEB, DIRP, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Brain Evolution and Behavior		
SECTION Unit for Research on Behavioral Systems		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 1.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A pronounced <u>micrencephaly</u>, characterized by a great <u>reduction</u> in the <u>neocortex</u> and <u>limbic cortex</u> of the <u>rat brain</u>, is induced by injecting pregnant females with <u>methylazoxymethanol acetate (MAM)</u> on the 15th day of gestation. The objective of this study was to determine if micrencephalic rats were capable of successfully inhabiting large, complexly structured environments while living as free-ranging social groups. As in previous studies it was demonstrated here that MAM-induced micrencephalics are capable of successful reproduction in restricted, laboratory cage environments. However, prior to this study it was not known if the entire mantle of cerebral cortex was required to enable a mammal such as the rat to cope successfully with the dynamic situations known to occur in free-ranging, quasi-natural, laboratory populations. Our results show that <u>micrencephalic rats</u> do not adjust to new social/environmental conditions as <u>quickly</u> as <u>controls</u>: Upon introduction to a new situation micrencephalic rats are <u>much more aggressive</u> and have about three times as many <u>wounds</u> as controls. Also, initially micrencephalic animals have <u>less reproductive success</u> than that of controls; in first litters micrencephalic females rear to the weaning age only 13 percent of the number of pups nurtured by control females. After a <u>prolonged</u> period of <u>adjustment</u>, micrencephalic females are about <u>50 percent</u> as <u>successful</u> as control females in rearing pups to the age of weaning. Both micrencephalics and controls are <u>influenced</u> by the <u>stress</u> resulting from increased population density, with the effect being more pronounced in the micrencephalics. At a population density of 16 adults per environment, control rats are only about 56 percent as successful, and micrencephalics about 28 percent as successful, as are equivalent rats housed at a density of eight adults per environment. Observations of other behaviors also indicate that the treated animals have <u>difficulties in coping with social and environmental complexity</u> especially upon initial exposure to new situations. A major conclusion based on results of this study is that adult rats handicapped by <u>arrested neocortical development</u> may require a longer period of <u>adjustment</u> to new social and environmental situations than experienced controls. </p>		

Project Description:

Other Professional Personnel: John B. Calhoun Chief, URBS LBEB NIMH
 Paul D. MacLean Chief, LBEB NIMH

Objectives:

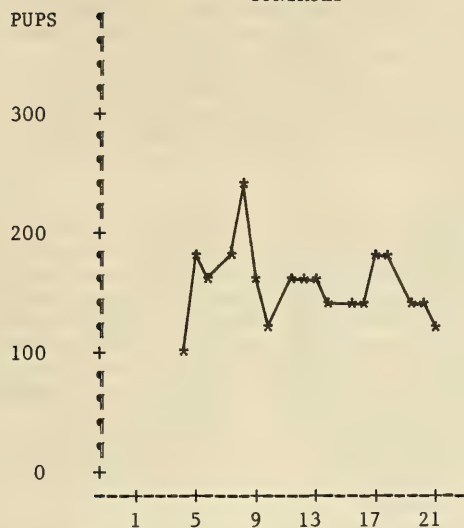
The object of this study was to determine if micrencephalic rats with a great reduction of neo- and limbic cortex are able to develop appropriate behaviors for surviving and successfully rearing young over an extended period while living as groups in complex environments.

Methods: The procedures for inducing micrencephaly in rats using Methylazoxymethanol acetate (MAM), and the eight environments in which MAM-treated groups were compared with groups of controls were described in Z01 MH 00847-01 and 02 LBEB. The rooms were of two different levels of structural complexity and the more complex rooms housed twice as many rats (eight bisexual pairs) as the less complex rooms. Thus there were two replicates of each MAM-treated versus control environmental complexity combinations.

All ninety-six rats in the eight rooms were surgically implanted with a passive resonator which enabled their activity to be monitored by a computer controlled data acquisition system. Data on reproductive and physical condition of adults, and the numbers, weight and condition of pups, were recorded during weekly surveys of all animals. The location of each adult's nest site, and the estimated age of all pups were recorded daily. Behavioral observations were made during daily inspection of each room, and at intervals from the viewing window in the ceiling of each room. During the latter observations, the occurrence of ultrasonic vocalizations and associated behaviors were recorded. Observational data, the weekly survey data, and daily inspection data were pooled to determine the social/reproductive structure within each environment. Reproductive performance of the rats in the environments was compared to that of bisexual pairs housed in standard laboratory cages. At autopsy, general condition, body weight, weights of spleen, kidneys, and adrenals, four measures of skull size, and number of placental scars in females were recorded for comparisons.

Major Findings: Of primary significance is that MAM-induced micrencephalic rats survive and produce young over an extended period while living in social groups in complex environments. However, their ability to successfully rear their young beyond the first few hours is less than that of controls, and is more reduced by increased population density. The number of pups alive and weighed at each weekly survey for the MAM-treated rats was only about one-half of the number of the controls during most of the study (Figure 1, top two graphs). This difference was not due to a failure to reproduce but was the result of the poor parental behavior of the MAM-treated rats in the social groups. The number of pregnancies, as indicated by postmortem count of placental scars, did not differ significantly between MAM-treated and controls; however fewer of the offspring of MAM-treated rats survived long enough to be counted. Blood on nesting paper in nests of females in post-partum estrus, and partially consumed bodies were taken as evidence that pups were cannibalized soon after birth. In other studies in this laboratory it has been shown that when rodents are in socially stressful situations their parental behavior toward newborns suffers. An indicator of social stress is the amount of aggression between males; in this

CONTROLS



MAM-TREATED

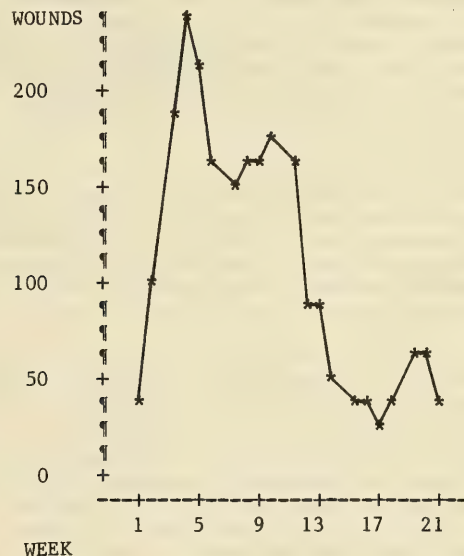
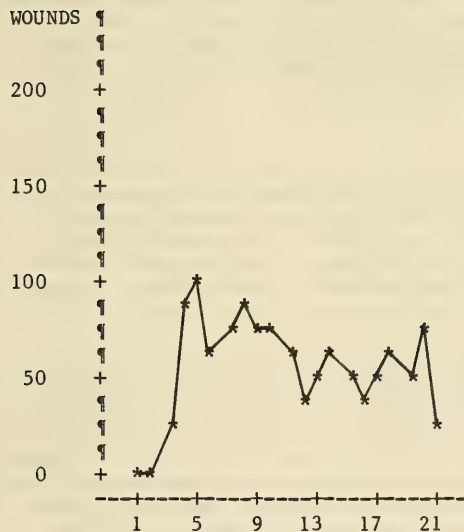
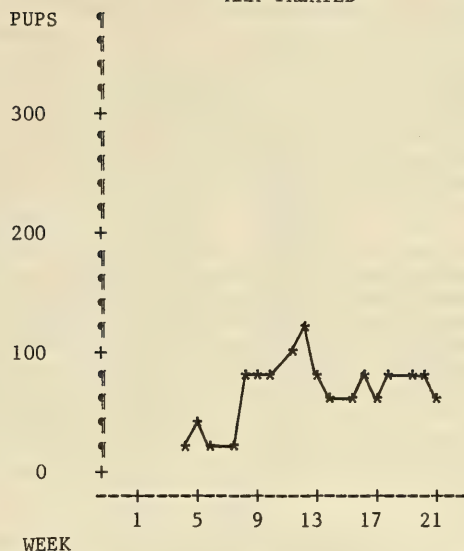


FIGURE 1. COMPARISON OF WOUNDING AMONG MALES AND THE NUMBER OF LIVING OFFSPRING IN THE TWO TREATMENT GROUPS

study, the number of wounds counted at each survey was taken as an index of the amount of aggression between males. The bottom two graphs in Figure 1 show that not only were the overall number of wounds greater among MAM-treated males, but, that during the first part of the study, when the survival of MAM-treated offspring was very low (especially the first litters), the inter-male aggression was very high. It is newborn and young pups which suffer most from poor parental behavior; the growth curves for surviving pups do not differ between the treatments, indicating that the MAM-treated rats are as capable as are controls of caring for pups more than a few hours old.

The reactivity of the MAM-treated and control rats to social stress can be seen in Figure 2. If we assume that a population density of eight pairs is more stressful than four pairs, then both the controls and the MAM-treated rats reacted to the increase in stress by carrying significantly fewer offspring to weaning age, and the MAM-treated rats were influenced more by the density/stress increase. Populations of eight pairs of MAM-treated rats weaned only 28% of the number of pups weaned by populations of four pairs compared to the controls in which the eight-pair populations reared 56% of the number of pups reared by those of four pairs. Also presented in Figure 2 are the numbers of pups weaned by single, caged pairs of both treatments. The caged pairs also reared more pups than the populations of eight pairs and slightly fewer than the populations of four pairs.

Although not statistically significant, the tendency for rats in small social groups of four pairs to rear more pups to weaning than single caged pairs, suggests that there is some advantage to living in small social groups; females in the small groups often raised their young communally so that the young were almost always in the presence of at least one adult female. The data suggest that living in a group of four pairs, as indicated by ability to carry young through to weaning, is less stressful than either living in a group of eight pairs or as an isolated bisexual pair.

When the data in Figures 1 and 2 are examined together it becomes evident that the micrencephalics took longer to adjust to new social/environmental conditions, and were more affected by higher density/social stress. However, it should also be noted that they eventually were able to successfully rear young, and became involved in less aggressive behavior, which suggest that the cortical areas in the forebrain have an important role in facilitating a rapid adaptation to new and stressful social and environmental conditions.

Direct observations of behavior were correlated with the survey, autopsy, and computer recorded activity data. Animals observed fighting had more wounds than those which avoided aggression, and, dominant, attacking individuals were scarred about the head and face whereas subordinate individuals, which fled from aggressive encounters, were scarred about the back, rump, and tail. Post-partum estrus, as evidenced by lordosis and mounting, was observed in females known to have given birth earlier in the day, and estrus behavior was observed reliably just prior to the darkest phase of the light cycle or birth days of litters. The latter was confirmed by the computerized activity monitoring system which showed significant increases in movement, especially by males following estrus females throughout the environment. Preliminary examination of ultrasonic vocalization data do not indicate any significant differences between adult MAM-treated and control rats.

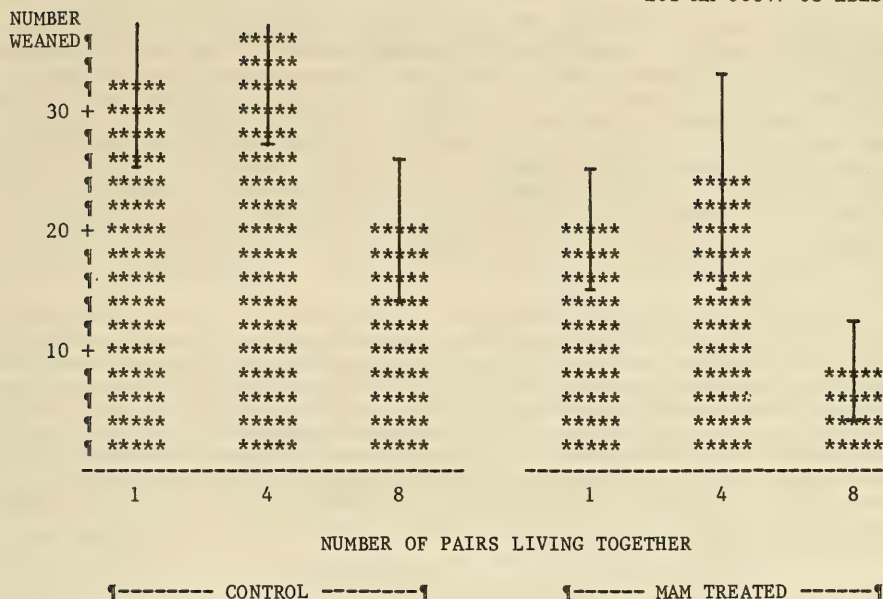


FIGURE 2. NUMBER OF PUPS WEANED PER FEMALE IN EACH OF THE
TREATMENT/DENSITY COMBINATIONS
(MEAN \pm 95% CONF. LIMIT)

From about one hundred days of age the MAM-treated rats of each sex were significantly lighter in weight than equivalent controls (about 88%). Therefore, spleen, kidney, and adrenal weights, and cranial measurements taken at autopsy were adjusted for differences in body weight before they were compared. Differences in adjusted weights were due to differences in body weight and to sex with females having heavier spleens and adrenals and lighter kidneys; the sex differences in organ weights probably reflect the greater physiological cost in reproduction to females. Cranial measurements when adjusted for body weight differences show that the MAM-treated animals have significantly smaller craniums than do controls (about 80%). Preliminary samples indicate that the total brain weight of the MAM-treated rats is about 60% of that of the controls.

Preliminary histological examination of the brains of siblings of treated animals indicates that the entire cerebral hemispheres are reduced by about 50 percent; the neocortex and limbic cingulate cortex show the greatest reduction in area and disruption in layering pattern. The two caudal cytoarchitectural areas of the cingulate convolution undergo greater reduction and disruption of the layers than the two rostral areas. The posterior neocortical areas also show greater reduction and disruption of the layers than the frontal areas. The hippocampus remains fairly well developed. The corpus collosum is absent except for the part above the septum. The thalamus is less reduced in size than one would expect in view of the loss of cortical tissue. The midbrain cerebellum, and medulla appear to be little affected.

Significance to Biomedical Research and the Program of the Institute:

This project involving experiments, in which the cortical development in rats is massively reduced, represents a model system for observing the effects of brain altering, teratogenic agents upon the behavior of adult, social animals. This research will also permit an assessment of the degree to which species typical behavior is regulated by cortical areas. Studies of individuals and groups of rats with experimentally induced micrencephaly can lead to a better understanding of the principles of how organisms, including humans, are able to eventually cope with environmental and social complexity, despite the mental disadvantage resulting from arrested brain development.

Future Course: The animals have been removed from the environments and the brains are being readied for histological examination. The comparison of behavioral and histological data will be conducted upon completion of the histological examination of the brains and the project concluded with the preparation of the results for publication.

Publications:

Burkholder, J.H., J.L. Hill, W.J. Vaughn, and H.E. Cascio. A broadband digitizing rat detector: Simultaneous recording from all sound frequencies in the range of rat ultrasonic vocalizations. Behavioral Research Methods and Instrumentation. 14: 571-518, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00849-01 LBEB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Resting time residence in a 7-generation population of house mice.		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) John B. Calhoun Chief, URBS, LBEB, DIRP, NIMH		
COOPERATING UNITS (if any) none		
LAB/BRANCH Laboratory of Brain Evolution and Behavior		
SECTION Unit for Research on Behavioral Systems		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Poolesville, Maryland 20837		
TOTAL MANYEARS: <div style="text-align: center;">2.2</div>	PROFESSIONAL: <div style="text-align: center;">1.0</div>	OTHER: <div style="text-align: center;">1.2</div>
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The analysis is just beginning of a 7-generation population of house mice (<u>Mus musculus</u>) that lived in an environment that was optimum for 200 adults. Population growth was regulated so that over eight successive 200 day periods the adult population formed a series of 16, 100, 200, 400, 800, 1600, 1600, 374 individuals. At optimum density, approximately 12 adults lived in each of the 16 similarly constructed areas (termed cells), of the habitat. During the 6th period only sufficient pups of the 7th generation survived to maintain the population at eight times optimum density. Only 2 pups survived to form the 8th generation. No pups survived during the 8th period when the population was reduced to 372 7th generation adults.</p> <p>We had anticipated that living at 4x optimum density would precipitate population extinction despite our efforts to maximize favorability of habitat conditions. Our long-range objective was to understand the process of <u>dissolution of organization of biobehavioral systems</u> existing in a <u>constant environment</u>.</p> <p>Places to which each mouse customarily retires define its <u>core residential range</u>. Local groups are comprised of individuals with overlapping core ranges. Each habitat cell contained 70 structurally unique numbered subspaces. Each of the 2500 mice was captured resting many times during its average 550 day life span. After random dispersal as juveniles, a period of core range stability usually ensues. Location of core range may shift 2 or more times during adulthood with intervening range instability. Algorithms and computer programs have been completed to define core range stability and instability and thus the periods of heightened stability of the entire population. Such analyses of resting-time residence are intended to provide a logical structure for analyzing our extensive data base of behavior observations.</p>		

Objective: To develop a strategy for determining the role of the structure of physical space on the course of the developing stability of a population of mice and on the disintegration of the patterns of interrelationships among individuals as severe overcrowding develops.

Our general theoretical orientation holds that much of early mammalian evolution revolves around patterns of movement through physical space, and the use of, and attachment to, components of the environment. Words and phrases such as home, home range, territoriality, and optimum group size reflect the consequence of adaptive adjustment to the physical environment. In a similar fashion, words and phrases such as separation anxiety, strange object reaction, withdrawal and anomie reflect some of the consequences of shift from customary relations within the physical environment.

Most mammals return to one of a few locations during periods of reduced activity. These places of resting time residence constitute a most restricted core residential range. We are developing a strategy for utilizing such resting time residences for our mice as the critical starting point for understanding the physics of negentropic-entropic changes (i.e. positive developmental vs negative disintegrative) in the relationships among individuals over the 7-generation history of the population.

See accompanying Systems Analysis flow chart.

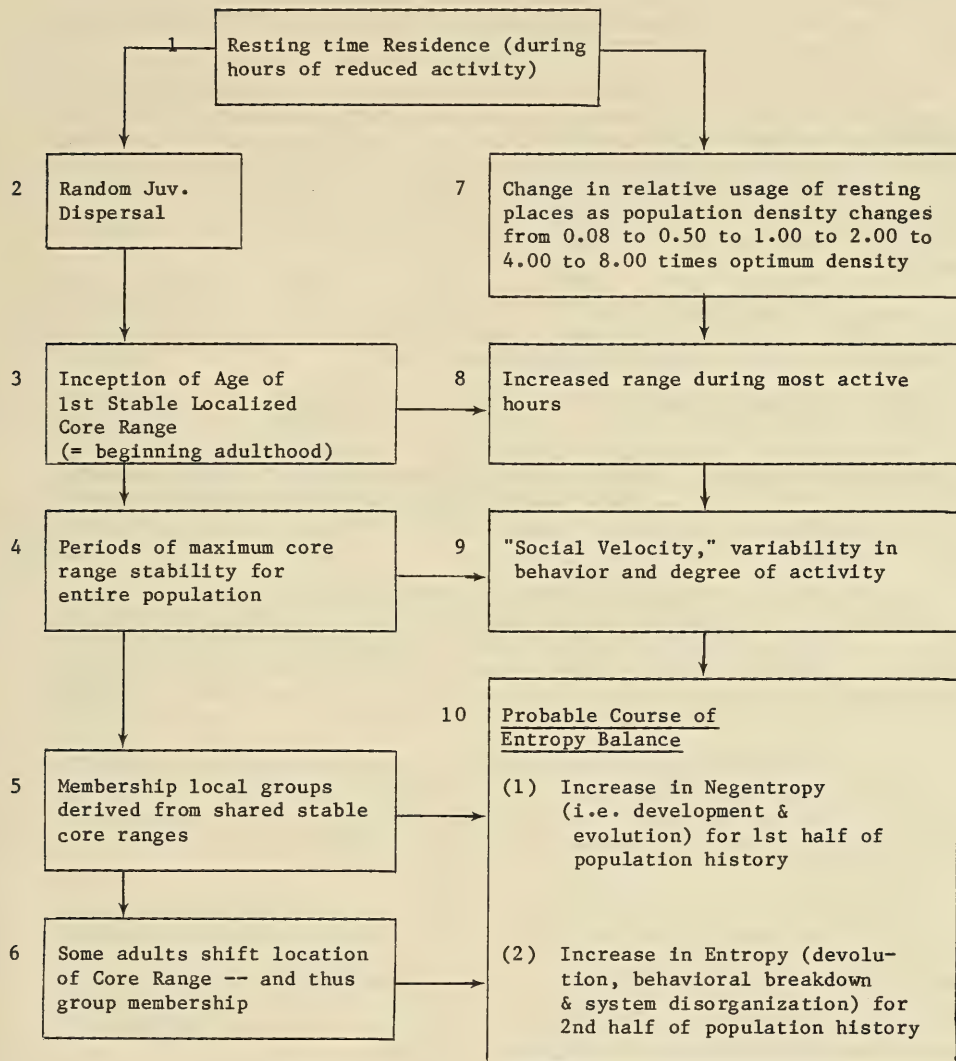
Success of this research has been made possible by full-time participation of James L. Hill, Visiting Associate, and Garrett A. Bagley, Computer Programmer, both of URBS, LBEB, DIRP, NIMH, as well as of dedicated support staff.

Methods: The general methodology and some tentative conclusions may be found in Z01 MH 00835-01 to 05 and 00848-01 LBEB. The "circular" habitat consisted of 16 pie-shaped spaces, termed cells. Each of the 16 cells included 70 numbered local places where one or more mice might be caught. Any weaned mouse had the locomotor capacity to move rapidly from any one of these locations to any one of the other 1120 locations. 2500 subjects lived into adulthood. The typical mouse lived for 80 weeks and was captured during 20 of these weeks during a time of day of very low activity-level. The exact spot of each capture was recorded along with the mouse's eartag I.D. number. Each such capture was placed on one of four computer files depending on the purpose of related data recorded. These four files were restructured and fused into a single file that permitted easier printed visual display of the life history of resting time captures.

A subset of 400 such displays was subjected to detailed examination in order to develop algorithms which could select start and termination of successive periods of stability and instability of resting time residence. Stability is defined as predominant resting time residence in a single cell.

Major Findings: Preliminary examination of the data indicate that random dispersal begins shortly after weaning, i.e. from about 30 days of age. The typical mouse usually does not settle down until after another 60 days of age. Thereafter most mice shift ranges two or three times during the central 400 days of their life. Various length episodes of range instability intervene between stable residential periods.

Strategy for Biobehavioral Systems Analysis
 Focused Outward from Resting Time Residence



The accompanying flow chart of a strategy for Biobehavioral Systems Analysis represents a major research accomplishment. It establishes efficient and cost effective guidelines for dissecting such living systems, discovering basic functional properties and integrating them to understand better how such a complex living system develops and then disintegrates in the absence of opportunities for non-genetic evolution. Steps 1 to 3 are briefly treated above. The remaining 7 analytical steps need further comment:

Step 4: Through steps 1 to 3 it will be possible by a single computer analysis to determine which weeks and days of adult life of each of the 2500 subjects are residentially stable or unstable. Thus a graph may be developed across the 1600 day history of the population as to what proportion of the adult population is residentially stable. Since the population history is divided into ca. 200 day next-generation-inception episodes, one may then identify the main period of residence stability within each such 200 day sample.

Step 5: Step 4 leads directly to identification of the major individuals which comprise the group membership of each of the 16 cells as distinct from floating, unsettled, components of the population.

Step 6: We do not yet know when adults are most likely to shift cell of residence within the 200 day generational cycle. Our previous studies suggest that such shifts of residences bring reduction of social status and are to regions of generally lower social class. Therefore we shall be particularly interested in following how such changes contribute toward greater systems entropy, toward disintegration of individual behavior and intra- and inter-group relations.

Step 7: When the population was quite low, during the first three generations before optimum density had been exceeded, the places where mice resided were quite different from the more exposed public space locations that were more used by later generations after crowding had developed. We suspect that this change in the usage of local spaces will be particularly useful in delineating the negentropy to entropy change over the history of the population.

Step 8: Extensive sets of observations were made on the behavior of 12 to 20 mice/cell each 200 days during the 8 daily hours of peak activity. Extent of range is much greater than the core residential range during these hours of increased activity. We will be particularly interested in the contact rate between neighboring groups as increased crowding ensues.

Step 9: The behavior observations of Step 8 provide the data base for calculating the level of "social velocity" of adult members of the population. Social velocity reflects the degree of motor activity and alertness, and the adequacy of behavior. Ranking members of an optimum sized group by social velocity reveals that (a) the difference in velocity between any two adjacent ranked members is a constant, (b) the difference of velocity of the lowest ranked individual from zero is this above constant.

Step 10: Theory, and results from certain of our prior studies, indicate that the sum of the social velocities of a population cannot exceed that which

characterized this population when it was composed only of optimum sized groups. Following the strategy of analysis through Step 9 will enable us to determine if we really can describe the decay of a population by a concise set of principles.

Significance to Biomedical Research and the Program of the Institute.

This study of mice suggests that it is possible to assess the mental health of any mammalian population, including humans, in terms of the capacity of its members to engage in customary or traditional patterns of behavior, or to develop new, more adaptive, ones. An advantage of this system's physics of behavioral action and interaction is that it provides a generation or two of warning as to whether the long-range history of the population will be one of negentropy enhancement (i.e. evolution) or increase in entropy (i.e., devolution or system degradation).

Proposed Course: To complete the strategy of Steps 1-10 of analysis outlined above. Each of the 10 steps encompasses several large-scale analyses.

Publications:

Calhoun, J.B.: The Built Environment and Information. Man-Environment Systems. 12: 183-184, 1982.

Calhoun, J.B. (Ed.): Environment and Population: Problems of Adaptation. New York, Praeger Publishing Co., 1983. 506 pp.

Honors:

November 1982. "In recognition of John B. Calhoun, Ph.D., whose life work has been an inspiration to all who aspire to a science of human behavior." Georgetown Family Center, Georgetown University, Washington, D.C.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00850-01 LBEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Cooperation induced modification of behavior in rats.

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

John B. Calhoun Chief, URBS, LBEB, DIRP, NIMH

COOPERATING UNITS (if any)

none

LAB/BRANCH

Laboratory of Brain Evolution and Behavior

SECTION

Unit for Research on Behavioral Systems

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Poolesville, Maryland 20837

TOTAL MANYEARS:

2.5

PROFESSIONAL:

1.0

OTHER:

1.5

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Two populations of rats (Rattus norvegicus) were studied for 18 months in complex, compartmentalized habitats to determine if acquisition of cooperative behavior could prevent the origin of behavioral pathologies which usually accompany crowding level densities. Members of the experimental population were required to learn two quite different and complex types of cooperative behavior, one to obtain water and the other to obtain food. Learning and maintaining these cooperative behaviors takes considerable time. Preliminary analyses indicate that the two sexes gain this time in quite different ways. Females in the two populations became pregnant in equal frequency. However, experimentals reared only 40% of their litters; the remainder disappeared very shortly after birth. This manner of "avoiding" rearing young provided time for the experimental females to learn and maintain cooperative behavior. Experimental males apparently gain the necessary time for learning cooperative behavior by reducing the frequency and duration of status interactions.

Strategies have been developed for obtaining precise measures of how much reproductive and status behavior time is utilized by experimentals in order to develop cooperative behavior. These measures are deduced from movements each rat makes between compartments of the habitat. Special technology records ca. 200 passages per rat, by its I.D., each day over its life span, for each of 80 rats.

These passages between compartments, in conjunction with extensive observations of status interactions, permit calculation of an index, termed "social velocity", which reflects behavioral competence, and degrees of participation in opportunities for behaving. This index should prove to be particularly useful in our analysis of how the experimental cooperative population moves in the direction of decreased entropy, that is to say, toward increased diversity, complexity, organization, adaptability and capacity to process information. These latter anti-entropy processes comprise increase in negentropy.

Objective: To develop a strategy of analysis which will reveal if, and in what way, the opportunity to acquire cooperative behavior by rats increases the level of negentropy of a biobehavioral system relative to its entropy.

Our general theoretical orientation holds that biological-genetic evolution is ultimately superseded by a cultural-learned evolution involving acquisition of cooperative roles which permit both increase of negentropy and population density without violating the inherent need for social contact rate equivalent to that which earlier occurred in closed optimum sized groups of 12 adults. Learning and maintaining new cooperative behaviors requires considerable time. This means that less time can be spent in certain of the more traditional behaviors. In order to help us learn how this new form of evolution takes place we are making analyses of time and kind of activity to determine what behaviors are reduced to permit acquisition of cooperative behaviors. Initial emphasis is placed on changes in reproductive behavior.

Methods: Details of the general methodology may be found in prior Z01 MH 00836-01 and 00848-06 LBEB. Two populations of 40 rats each were studied for 18 months in very complex compartmentalized habitats. Each 21 days every subject was caught and examined and detailed visual observations were made of each population. Visual observations, made through windows in the ceilings of the habitats, were facilitated by color coded markings on the fur of subjects which gave each rat a unique I.D. number. Each rat also carried a passive resonator implant that permitted continuous monitoring of its movements through the compartmentalized environment as it passed through detection "PORTALS" at the openings between compartments. Experimental subjects were required to learn two quite different types of cooperative behavior, separate ones for obtaining food and water.

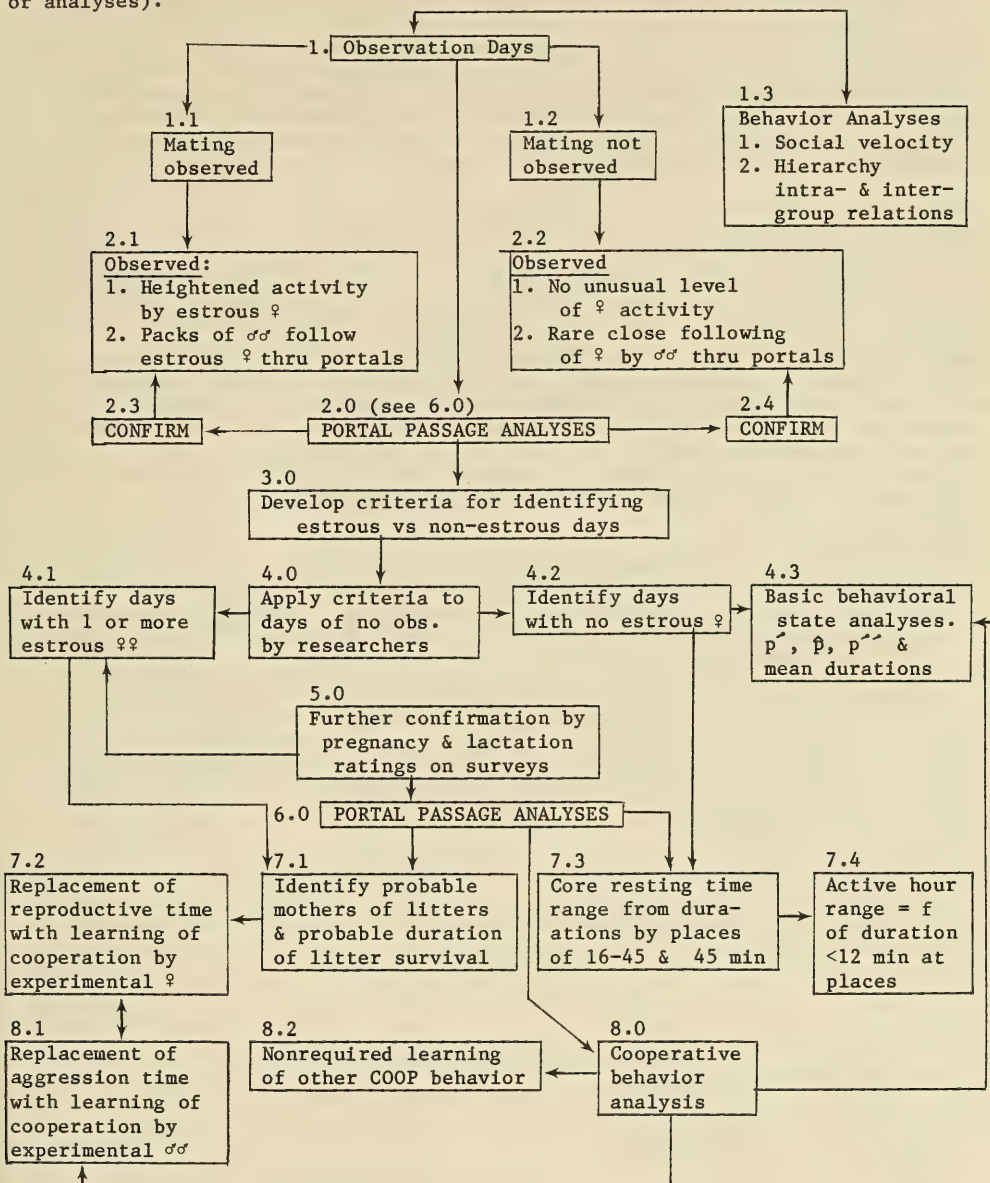
Garrett A. Bagley, Computer Programmer, URBS, LBEB, DIRP, NIMH, has managed the maintenance of the complex behavior computer recording system and has conducted all needed computer analyses.

The accompanying systems analysis flow chart is being followed to increase the effectiveness of our analysis of these two biobehavioral systems.

Results: On days of estrus, frequently the immediately post-partum estrus, females become unusually active and range more widely. Soon they are followed by packs of 3 to 12 males. Each time an estrous female goes through a portal she is very rapidly followed by a pack of males. This increase in estrous day activity and the heightened male following is verified by analysis of portal passages. Criteria thus established enable precise recognition of estrus on days when there were no visual observations.

Rats on cooperative behavior demand (COOP experimentals) had as many recorded pregnancies as did the controls. However, COOP females reared only 40% as many litters as controls. Thus it appears that COOP females gain time for learning cooperative behavior by not having to rear as many litters. We do not know how this early infanticide occurs.

Strategy for Biobehavioral Systems Analysis Starting with Focus on Changes in Reproductive Behavior (Numbered steps designate sequentially necessary procedures or analyses).



Significance to Biomedical Research and the Program of the Institute.

Engaging in cooperative behavior is the hallmark of culture and civilized life. These studies on rats should help us understand the origin of the greater than exponential rate of increase in cultural evolution among humans which began some 40 millennia ago. More importantly the knowledge of the process of acquiring and maintaining cooperative behavior should become particularly relevant during the 1975-2175 evolutionary transition when cooperation in enhancing information metabolism is likely to become much more important (See prior Z01 MH 00838-04 LBEB and Z01 MH 00848-01 LBEB).

Future Course: Determination of what behavior males modify in order to learn and maintain cooperative behavior. Preliminary analyses of observations suggest that males find time for cooperation by reducing frequency and duration of aggressions. Algorithms defining chases and avoidances permit recognition by the computer of this kind of status interaction from the timing of portal passages of any two males. Then we will examine the extensive files of observed social behavior and coordinate these data with portal passages to obtain indices of "Social Velocity," here used as a reflection of degree of motor activity or alertness, and competence to execute adaptive behavior. In essence the index of Social Velocity permits ranking the members of a population on a negentropy-entropy scale, and the sum of the indices for a population for sequential times will indicate the differences between the histories of the control population and the cooperative experimental population.

Publications, Honors: See Z01 MH 00849-01 LBEB

Klopf, A. H.: The Hedonistic Neuron. Washington: Hemisphere, 1982, xvi + 140 pp.

(Note: The central theory of this book was first presented by Dr. Klopf at the October 27-28, 1972 review of the Laboratory of Brain Evolution and Behavior by the NIMH Board of Scientific Counselors. The title of Dr. Klopf's presentation was: "Heterostasis, not homeostasis: A new paradigm for investigating brains and societies.")

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00881-27 LCM
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Intermediary Energy Metabolism in Mammalian Brain		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Elaine E. Kaufman, Research Chemist, Laboratory of Cerebral Metabolism, NIMH		
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION National Institute of Mental Health, Bethesda, Maryland, 20205		
TOTAL MANYEARS: <div style="text-align: center;">3.75</div>	PROFESSIONAL: <div style="text-align: center;">2.25</div>	OTHER: <div style="text-align: center;">1.5</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p> This report covers three projects. The major project is a study of the biosynthesis, mode of action, and metabolism of <u>γ-hydroxybutyrate</u> (GHB). Biological factors which regulate the enzyme that converts GHB to succinic semialdehyde (SSA) have been studied. Both <u>in vivo</u> and <u>in vitro</u> studies on the conversion of <u>putrescine</u>, a known precursor of GABA, to GHB are underway. The conversion of arginine and β-hydroxybutyrate to GHB are also being studied. </p> <p> A study concerning the effect of elevated levels of the ketone body, <u>acetoacetate</u>, on the formation of <u>GTP</u> and <u>cGMP</u> in the brain has been completed. </p> <p> A third study concerned the <u>in vivo</u> incorporation of <u>2-deoxyglucose</u> into <u>glycogen</u>. This study has also been completed. The results indicate that 2-deoxyglucose is incorporated, but only to a very small extent. </p> <p> The goals of this project are 1) to elucidate the role of naturally occurring GHB in the brain and in peripheral tissues; 2) to understand the biochemical basis for the profound pharmacological actions of GHB; and 3) to investigate the pathways of biosynthesis and degradation as well as the normal metabolic factors and pharmacological agents which control these pathways. </p>		
(815)		

OTHER INVESTIGATORS:

Thomas Nelson
Louis Sokoloff

Staff Fellow
Chief, Lab. of Cerebral Metab.

LCM NIMH
LCM NIMH

Project Description:

This project continues into its fourth year of studies on the origin, mode of action, and metabolism of GHB, a compound which is a normal constituent of mammalian brain, and which may function as a neuromodulator or possibly as a neurotransmitter. When administered in pharmacological doses, GHB produces a flattening of the EEG, a reversible trance-like state, and a profound depression of cerebral glucose utilization. This compound is being used clinically in Europe as an anesthetic adjuvant. Preliminary trials indicate that it is effective in the treatment of narcolepsy and may be of use in the treatment of stroke. Recently it has been reported that GHB can block the myoclonic jerks produced by injecting mice with muscimol. This suggests that GHB may be of value in the treatment of Lance-Adams Syndrome and other forms of myoclonic seizures. Because GHB is a compound which is used clinically, and because it is a naturally-occurring compound, we feel that it is important to understand not only pathways of metabolism of GHB, but also the factors which control these pathways.

An enzyme capable of metabolizing GHB has been found in tissues such as liver and kidney as well as in brain. This enzyme, an NADP⁺-linked alcohol oxidoreductase which interconverts GHB and SSA, has been purified from hamster liver and partially purified from hamster brain. Throughout this report this enzyme will be referred to as GHB dehydrogenase so that the catalytic activity of interest to this laboratory will be clear. Studies completed on GHB dehydrogenase include substrate specificity, enzyme kinetics, molecular weight determination, and inhibition studies with the anticonvulsant agents, diphenylhydantoin, amobarbital, and valproate.

This project is directed at understanding the role of naturally-occurring GHB as well as the metabolic pathways involved in its synthesis and degradation. Since the compound is also used as a drug, other important questions arise concerning its pharmacological actions. In addition to the pharmacological effects already mentioned (flattening of the EEG, trance-like state, and depression of cerebral glucose utilization) it has recently been reported that GHB increases the survival of animals which have been made hypoxic. The authors report that the blood and brain tissues of animals which have received GHB showed marked changes in the levels of some intermediates in carbohydrate metabolism as well as changes in levels of high energy phosphate compounds. These studies have led to the proposal that GHB may be useful in the treatment of stroke.

We have recently found that GHB dehydrogenase also catalyzes the reduction of D-glucuronate to L-gulonate. The reduction of D-glucuronate to L-gulonate is an important step in the pathway leading from UDP-glucose to L-ascorbate or to L-xylulose and the pentose pathway. We have recently investigated the effect of both L-gulonate and of D-glucuronate on the rate of oxidation of GHB. In the presence of limiting concentrations of NADP⁺ or with limiting concentrations of NADP⁺ and inhibitory concentrations of NADPH, D-glucuronate can increase the

NADP^+ and inhibitory concentrations of NADPH, D-glucuronate can increase the rate of oxidation of GHB by up to eightfold. Since GHB dehydrogenase is a soluble enzyme found in the cytosol, and since the cytosol of most tissues contains low concentrations of NADP^+ as well as relatively high concentrations of NADPH, D-glucuronate may be an important factor in controlling GHB metabolism in vivo.

The physical and chemical properties of GHB dehydrogenase have been investigated. Treatment of GHB dehydrogenase with a reducing agent such as dithiothreitol produces marked inhibition of activity. This inhibition can be at least partially reversed by the addition of an oxidizing agent such as hydrogen peroxide. We have recently found that complete reversal of the inhibition by dithiothreitol can be achieved by the addition of oxidized glutathione. This suggests that the activity of GHB dehydrogenase may be regulated by the ratio of oxidized to reduced glutathione.

A study on the purification and characterization of GHB dehydrogenase has been completed and published. This enzyme has been purified 300-fold and found to exhibit a single band on gel electrophoresis. The protein is a monomer with a molecular weight of $\sim 31,000$ daltons. GHB dehydrogenase is inhibited by many of the anticonvulsant agents, such as amobarbital, diphenylhydantoin, and valproate. Pyrazole, the specific inhibitor of NAD^+ -linked alcohol dehydrogenase (ADH), does not inhibit this enzyme. Diethyldithiocarbamate, the reduced form of the drug antabuse, as well as KCN, both metal-chelating agents, inhibit GHB dehydrogenase. This suggests that the enzyme may contain either copper or zinc as an integral part of the protein.

We now have evidence that GHB dehydrogenase is identical to D-glucuronate reductase and that the ability to oxidize GHB to SSA represents a new activity for D-glucuronate reductase. The unusual attribute of GHB dehydrogenase is its ability to couple these two reactions (the reduction of D-glucuronate to L-gulonate and the oxidation of GHB to SSA) and in so doing to alter markedly the kinetic constants for GHB, and the cofactors, NADP^+ and NADPH. A detailed study of the kinetic constants in both the coupled reaction and in the uncoupled reaction has been completed. This study showed that the kinetics of the uncoupled reaction proceeded by a Rapid Equilibrium Random BiBi mechanism. The alteration of the kinetic constants for GHB and the cofactors, NADP^+ and NADPH, brought about by coupling the oxidation of GHB to the reduction of D-glucuronate are of sufficient magnitude to make it possible to propose that this reaction may proceed under conditions approaching those which exist in the whole tissue. This is of significance since the catabolism of GHB has been shown to proceed through SSA to succinate and the citric acid cycle. In this pathway, the oxidation of GHB to SSA would be the first step. Any acceleration or inhibition of this reaction would be reflected in altered tissue levels of GHB.

The effect of a number of biological intermediates on the oxidation of GHB has been studied. The citric acid cycle intermediates succinate, pyruvate, α -ketoglutarate and oxaloacetate, the ketone bodies, acetoacetate and β -hydroxybutyrate, and the products arising from the transamination of phenylalanine such as phenylacetate were all inhibitory to varying degrees. The α -keto acids, α -ketoglutarate and oxaloacetate, were among the most inhibitory compounds. However, when the α -keto group was replaced by an α -amino group the resulting

amino acids, L-aspartate and L-glutamate, either did not inhibit or produced a slight stimulation as did L-alanine and GABA.

A detailed study of the effect of pH on the kinetics of both the simple (uncoupled) oxidizing GHB to SSA and on the coupled (+ D-glucuronate) oxidation of GHB has been carried out. Both V_{\max} and K_m were found to vary with pH. This resulted in a marked change in the pH_{optimum} for this reaction when the velocity was measured at low concentrations of GHB rather than under V_{\max} conditions. As the concentration of GHB approached physiological levels the pH_{optimum} shifted from pH 9 (V_{\max} conditions) to a more physiological pH (7.0 for the coupled reaction at 0.5 mM GHB and 7.5 for the uncoupled reaction at 1.0 mM GHB).

Endogenous inhibitors of GHB dehydrogenase.

The finding that a number of biological intermediates, namely the transamination products of L-phenylalanine and of the branched-chain amino acids are good inhibitors of the purified GHB dehydrogenase led to the prediction that these compounds might also inhibit the in vivo oxidation of GHB and thereby result in an increase in the tissue levels of GHB. This prediction has been tested. Measurement of tissue levels of GHB after infusion of α -ketoisocaproate (the transamination product of leucine) showed a twofold increase in kidney and muscle but only a 23% increase in brain. Infusion of phenylacetate (a metabolite resulting from phenylalanine oxidation) gave a twofold increase of GHB in brain with a small decrease in the levels in kidney and muscle. This last effect was unexpected, and we are currently investigating the possibility that phenylacetate not only affects GHB dehydrogenase but also some enzyme in the biosynthetic pathway which functions in peripheral tissues. These studies are of significance since phenylacetate and α -ketoisocaproate are compounds which are known to accumulate in phenylketonuria and in maple sugar urine disease.

Drugs such as salicylate and valproate are also good inhibitors of the purified enzyme. Valproate has already been shown by Snead et al. J. Neurochem. 19:47-52 (1980) to lead to increased brain levels of GHB. Recent studies in this laboratory have shown that salicylate (I.P.) will lead to a twofold increase in GHB levels in both brain and kidney.

Earlier work on the in vitro synthesis by GHB from GABA with intact brain mitochondria demonstrated that the rate of synthesis under anaerobic conditions was at least twice as fast as under aerobic conditions. When rats were exposed to 5.6% oxygen for a period of 2 hours the concentration of GHB in whole brain was increased threefold and in kidney, twofold.

These in vivo experiments have allowed us to test the results of our in vitro work on the purified GHB dehydrogenase and on the mitochondrial biosynthetic system. They have also contributed to our understanding of both the pathways of synthesis and degradation and of some of the factors which regulate these pathways.

Antibody. The purified $NADP^{+}$ -linked GHB dehydrogenase, which interconverts GHB and SSA, has been used to raise antibody in New Zealand white rabbits. The antibody has been purified, and when used in conjunction with Protein A sepharose columns is capable of quantitatively removing GHB dehydrogenase from

tissue extracts. The antibody has made it possible to demonstrate that GHB dehydrogenase represents 85-90% of the NADP⁺-linked GHB oxidizing activity in brain and kidney from birth to maturity. Furthermore, it has been possible to show that the remaining enzymatic oxidation of GHB is not inhibited by valproate and phenobarbital despite their marked inhibitory effect on GHB dehydrogenase. The antibody will be used as a probe in the study of the different oxidoreductases which are involved in the synthesis and degradation of GHB. The antibody will be used to show whether the various endogenous inhibitors of GHB dehydrogenase can also control the rate of GHB synthesis. This antibody has allowed us to confirm the identity between the NADP⁺ linked GHB dehydrogenase of brain, liver, and kidney. It should prove useful in answering questions concerning the subcellular localization of the biosynthetic as well as the degradative enzymes.

GHB levels in organs. The survey of rat organs for GHB reveals that GHB is widely distributed throughout all the organs which have been examined; its concentration in rat organs is shown in the accompanying table. Concentrations are expressed in nanomoles per gram of tissue.

ORGAN	BRAIN	HEART	KIDNEY	LIVER	LUNG	MUSCLE	BROWN FAT	WHITE FAT	BLOOD
Mean GHB	2.29	12.4	28.4	1.4	1.5	10.2	37.4	0.42	0.55
Std. Error	0.13	1.9	4.6	0.3	0.2	1.6	2.1	0.27	0.27
Number	36	36	36	36	36	36	100	7	12

These findings suggest that GHB plays some role in many, if not all, of the organs in the body. Until those results were obtained, it had been assumed that the most likely function of GHB was to modulate dopaminergic neuronal activity. Now additional functions will have to be sought for GHB in non-neural tissues, and these new functions may also have relevance to the brain. The most likely precursors for GHB were thought to be glutamic acid, γ -aminobutyric acid, and SSA, since experimental evidence shows that each of these molecules is capable of contributing carbon atoms to the skeleton of GHB. The finding of high levels of GHB in tissues such as kidney, which lack the means of decarboxylating glutamate to form γ -aminobutyrate, suggests that there must be other sources for GABA formation than glutamic acid. The most likely precursor may be putrescine or one of its metabolites.

Spectrophotometric Assay.

A new spectrophotometric assay for the detection of GHB has been devised. It is based on the use of the purified NADP⁺-linked GHB dehydrogenase and the synthetic co-factor, 3-acetylpyridine-adenine dinucleotide phosphate (APTPN⁺). The substitution of the synthetic co-factor shifts the equilibrium of the reaction $\text{GHB} + \text{APTPN}^+ \rightarrow \text{SSA} + \text{APTPN}^+ + \text{H}^+$ far to the right so that the GHB may be estimated by the amount of reduced co-factor which has been produced. We anticipate that this assay will prove useful in the estimation of blood and tissue levels of GHB.

Developmental Study.

A study has been completed in which we have determined both GHB levels and soluble cytosol NADP⁺-linked GHB dehydrogenase activity in several organs of developing rats from the late fetal stage to 20 postnatal days. GHB

concentrations in the newborn rat brains and livers are two to three times higher than they are in the adult. The concentration of GHB gradually decreases over the first 20 days of life to adult levels. GHB levels in the kidney rise from a lower level in the newborn period to attain the high level characteristic of the adult by 20 days. The enzymatic activities, on the other hand, tend to increase from the time of birth to 20 days in all the tissues, including brain.

This pattern of developmental changes is of special significance since previous widely accepted studies by other workers have suggested that GABA is the precursor for GHB in the brain. A soluble cytoplasmic enzyme has been implicated in the reduction of SSA in the cytosol to form GHB. It is known that during the same period of development in the rat both GABA transaminase and glutamate decarboxylase activity are low at birth and increase by some tenfold. These findings are consistent with a rate of GABA production which is low in the newborn and increases with age, while our present study shows GHB, a putative metabolic derivative of GABA, is highest at a time when GABA synthesis is low and falls as the rate of GABA synthesis increases. These findings suggest that GHB formation in the perinatal brain may involve precursors other than glutamate and possibly other than GABA.

Current experiments in this laboratory are being conducted to determine whether putrescine contributes significantly to GABA and GHB synthesis in the newborn rat brain. The ornithine decarboxylase inhibitor DFMO (difluoromethyl), and the monoamine oxidase inhibitor pargyline, have no effect on GHB synthesis in the adult brain. If the relative contribution of putrescine to GABA synthesis in the immature brain is large, then administration of these inhibitors to rat pups should produce significant falls in the GHB level in their brains.

Precursor for GHB. Efforts are currently underway to work out the biosynthetic pathway for GHB. We have used three main approaches to this problem. In the first, a number of carbohydrate intermediates, amino acids, and fatty acids were tested in an in vitro system for the ability to produce net synthesis of GHB. This has been a general survey of a number of possible precursors for GHB.

The second approach was similar to the first except that radioactive compounds were used in order to determine whether the compounds which gave rise to GHB in the first part actually contribute carbon atoms to the GHB molecule.

The third approach consists of in vivo experiments in which a radioactive precursor is injected and GHB is then isolated from the various tissues to determine whether the putative precursor has been incorporated in the GHB pool.

Results from the general survey of precursors, and preliminary work with radioactive compounds (in vitro), indicate that D,L- β -hydroxybutyrate and citrate are both capable of stimulating the formation of GHB, and of contributing carbon atoms to the skeleton of GHB. However, in the crude system of cell homogenate there is such a large amount of endogenous precursor or precursors that the specific activity of the GHB is reduced to about 10% of the specific activity of the precursor. The endogenous precursor is found in high concentration (up to 1 mMol/gr of kidney) in the soluble fraction of mitochondria from fed rats. Such high concentrations of endogenous precursor make it unlikely that β -hydroxybutyrate or citrate serve as the major precursor.

While β -hydroxybutyrate does not appear to be a major precursor, its stimulating effect on the synthesis of GHB from endogenous precursors in the soluble mitochondrial fraction is quite marked. In the presence of NAD^+ a concentration-dependence existed between D,L- β -hydroxybutyrate and GHB formation. Substitution of NADP^+ as the cofactor resulted in large amounts of endogenous substrate being converted to GHB even in the absence of D,L- β -hydroxybutyrate, though the inclusion of D,L- β -hydroxybutyrate resulted in even greater synthesis of GHB.

We infer from these results that there is a large pool of precursor which must be converted by an NADP^+ -linked enzyme to an intermediate. This, in turn, can be converted to another intermediate by an oxidative step which ultimately leads to SSA. The final reductive step would utilize the NADPH (generated at the NADP^+ -linked oxidation) or the NADH which could be generated in the presence of D,L- β -hydroxybutyrate. These results could be explained if putrescine is serving as the precursor. While putrescine added to kidney homogenate does not stimulate GHB synthesis, putrescine in the presence of an acetylating system does result in GHB synthesis. It is known that N-acetyl putrescine can be metabolized to GABA. The steps involve the oxidation of N-acetyl putrescine to N-acetyl γ -aminobutyraldehyde by a monoamine oxidase, followed by the further oxidation of the N-acetyl γ -aminobutyraldehyde to N-acetyl GABA by an oxidoreductase. This oxidoreductase in rat kidney appears to be NADP^+ -dependent, though a similar dehydrogenase in *Pseudomonas* which requires NAD^+ . NADP^+ would allow the N-acetyl γ -aminobutyraldehyde to be oxidized to N-acetyl GABA. After deacylation to form GABA the resulting SSA would be reduced to GHB in the presence of the NADPH which had been generated at the oxidative step. The stimulatory effect of D,L- β -hydroxybutyrate, and citrate (other than that deriving from their conversion to GHB), and that of other compounds such as ethanol, probably results from the generation of NADH during their oxidation. This NADH would then allow preexisting SSA derived from GABA and N-acetyl GABA to be reduced to GHB, though it does not permit the formation of SSA from N-acetyl butyraldehyde.

Pargyline and D,L- α -difluoromethyl ornithine have been administered to mature rats in order to demonstrate those organs in which ornithine and putrescine may serve as substantial precursors of GHB.

The following table summarizes the preliminary results. Concentrations are in nanomoles per gram of tissue \pm the SE.

TREATMENT	ORGAN		
	<u>Brain</u>	<u>Kidney</u>	<u>Muscle</u>
Saline (n=6)	2.74 \pm 0.42	38.5 \pm 3.6	21.9 \pm 3.0
DFMO (n=5)	3.0 \pm 0.40 (n=4)	21.1 \pm 3.2	18.6 \pm 3.1
Pargyline (n=4)	2.63 \pm 0.1	23.6 \pm 3.7	19.4 \pm 2.8

The results suggest that putrescine and ornithine may be the major precursors for GHB in kidney. In brain the lack of effect in the adult can easily be explained by the extremely large contribution of glutamate to GABA formation; consequently the very small portion of GHB derived from putrescine

would be obscured. The lack of effect in muscle suggests that ornithine and putrescine may not be important precursors for GHB in this tissue.

The irreversible GABA transaminase inhibitor Vinyl GABA will be used to determine whether there are other precursors for GHB which do not enter the final common pathway through GABA and SSA.

¹⁴C-Labeled putrescine has been used in a simple homogenate of brain and kidney to demonstrate that it can contribute carbon atoms to the GHB skeleton. As might be expected from the inhibitor study, the specific activity of the [¹⁴C]GHB was much higher in the kidney than in the brain. The converse was true of [¹⁴C]GHB derived from [¹⁴C]glutamate.

The kidney appears to be able to use arginine as a precursor for GHB synthesis (as judged by the net synthesis of GHB which is dependent on arginine). An acetylating system inhibits arginine's stimulatory effect upon GHB synthesis, thus suggesting that arginine may not be converted to putrescine in this pathway. Prokaryotic organisms are capable of converting arginine to GABA in a series of reactions which do not lead to putrescine. It may be that these enzymes are active in mammalian kidney and allow a similar conversion of arginine to GABA.

Thus far it appears that the major portion of GHB is derived from GABA. In brain, GABA is formed from glutamate through the GABA shunt pathway; in other tissues preliminary evidence suggests that polyamines or arginine may serve as the GABA precursor. The synthesis thus may be linked to the citric acid cycle, to the urea cycle, and to the polyamines which appear to play some regulatory function within the cell nucleus. The degradation of GHB is most likely accomplished by glucuronate reductase under circumstances which require a stoichiometric oxidation of aldehydes such as D-glucuronate to occur. Furthermore, the degradation of GHB appears to be controlled by several naturally-occurring α -keto acids, and phenyl ethyl alcohols which have recently been shown to inhibit D-glucuronate reductase. Pharmacological levels of GHB have been shown in this laboratory to produce a striking reduction of glucose utilization by the brain. Taken together these findings suggest that GHB might serve as some form of intracellular messenger, perhaps linking both protein and carbohydrate metabolism with nuclear events.

Effect of Naloxone on the Pharmacological Action of GHB.

It has been reported that naloxone can reverse some of the pharmacological effects of GHB, i.e., the effect on EEG charges, the accumulation of dopamine in the nigrostriatal pathway and the behavioral effect. Investigations in this laboratory of naloxone as an antagonist of GHB revealed that the effect of GHB on cerebral glucose utilization could be partially reversed in selected regions of the central nervous system. It was also observed that naloxone affected the drop in body temperature caused by a pharmacological dose of GHB. This aspect of the GHB project has been completed and a report of this work is in press.

Proposed Course.

This project will continue on the course which has been outlined. It will include: 1) further kinetic studies with GHB dehydrogenase; 2) determination of the presence or absence of metal in this enzyme; 3) in vivo studies to determine

the effect of various drugs, normal metabolites and alterations in physiological state on the metabolism of GHB; 4) investigation of the interaction of GHB with other pathways of carbohydrate metabolism; 5) developmental studies of GHB and a GHB dehydrogenase in brain and other tissues; 6) study of the biosynthetic pathway for GHB. If putrescine and arginine are the precursors of GHB it will be important to determine whether GHB has an effect on protein synthesis. 7) In the coming year the effect of GHB at the cellular level will be examined. This will include the effect of GHB on cyclic nucleotides, on the translocation of calcium and other ions, and in protein synthesis.

PROJECT #2. Effect of Ketones on Cerebral Metabolism.

This laboratory has had a continuing interest in ketone body metabolism in normal as well as in pathological states. D- β -Hydroxybutyric acid dehydrogenase catalyzes the reversible interconversion between D- β -hydroxybutyrate and acetoacetate, the two ketone bodies found primarily in the liver when fatty acid utilization is increased, as, for example, in diabetes, starvation, high-fat diet, and various states leading to ketosis. Acetoacetate is the primary ketone body formed from fatty acid catabolism in liver and muscle, and it is converted to D- β -hydroxybutyrate by the action of β -hydroxybutyrate dehydrogenase. Once formed, however, D- β -hydroxybutyrate cannot be used directly in any of the tissues; it must first be converted back to acetoacetate by the action of the same enzyme. This raises the interesting question of what role this enzyme plays. Why does the body not utilize the directly-formed acetoacetate rather than convert it first to D- β -hydroxybutyrate only to oxidize it back to acetoacetate again to utilize it? There is evidence in the literature to suggest that excessive concentrations of acetoacetate in the tissues may be toxic, particularly in brain which has recently been shown to utilize ketone bodies in direct proportion to their levels in the blood. The possibility exists that the role of the enzyme is protective, namely, to keep the acetoacetate concentrations low by storing it, in a sense, as D- β -hydroxybutyrate. It is noteworthy that there are several metabolic conditions characterized by ketosis and coma. Diabetic coma is one example, and recently Reye's Syndrome has been described. This is a frequently fatal disease of childhood which occasionally follows a viral infection, particularly influenza. It is characterized by fatty degeneration of the liver, ketosis, and coma.

Examination of the metabolic pathways and the enzymes responsible for the metabolism of ketone bodies in brain suggests possible mechanisms by which forced excessive use of ketone bodies in brain may lead to depressed cerebral functional activity. The ketone bodies enter the Krebs Cycle after their conversion to acetoacetylCoA, which in the presence of succinyl CoA-acetoacetate CoA transferase reacts with succinyl CoA to form acetoacetyl CoA. For every acetoacetate molecule thus metabolized, there is one succinyl CoA degraded. This reaction thus competes with the reaction catalyzed by succinic thiokinase which synthesizes a molecule of GTP for every molecule of succinyl CoA which is converted to succinate. This diversion of succinyl CoA would be expected to deplete mitochondrial GTP, which in turn might be expected to interfere with cyclic GMP synthesis. There is now a considerable body of evidence that cyclic GMP is the second messenger in the action of the neurotransmitter,

indeed suggest that in diabetic acidosis there is a depression of cyclic GMP in brain.

During the past year this project has received considerable attention. Biochemical studies have been carried out on the brain and blood of four groups of animals (adult male Sprague-Dawley rats): control animals, diabetic animals (diabetes induced by streptozotocin injection), diabetic animals which received insulin, and fasted animals. These animals were monitored for blood glucose and ketone bodies as well as active physiological and biochemical parameters. Brain levels of phosphocreatine, ATP, GTP, GDP, and cyclic GMP were measured and correlated with blood levels of acetoacetate, β -hydroxybutyrate, lactate and glucose. In the diabetic animals there was good correlation between the blood level of total ketones and C-GMP in brain ($P = 0.002$) and also between blood β -hydroxybutyrate and C-GMP in brain ($p = 0.0024$) in spite of the fact that the GTP levels in the control and diabetic animals were almost identical. This suggests that there may be some compartmentation of GTP.

Projected Course.

We plan to examine GTP and other metabolites in subcellular fractions of brain following infusion of ketone bodies.

PROJECT #3. Incorporation of 2-Deoxyglucose into Glycogen.

The investigation of 2-deoxyglucose incorporation into cerebral glycogen was not terminated as stated in the Annual Report of 1981-1982. Further experiments were required in order to obtain unambiguous and definitive experimental proof that the radioactivity which had been found in the glycogen fraction was indeed covalently bound to glycogen.

During the past year published reports indicated that ^{14}C - and ^3H -labeled 2-deoxyglucose are found in close association with typical glycogen granules seen on electron micrographs. This finding suggests that some of the 2-deoxyglucose-6-phosphate is being further metabolized to glycogen, and indeed reports now claim that perhaps up to 10% of the 2-deoxyglucose administered to the mouse, the leech, and the snail may be converted to glycogen. None of the reports contained vigorous proof that the label which appeared to be incorporated into glycogen was actually present as deoxyglucosyl units rather than as a deoxyglucose-6-phosphate or 2-deoxyglucose adsorbed onto the surface of the glycogen.

Experiments performed in this laboratory during the past year confirm that 2-deoxyglucose is incorporated into glycogen as glucosyl units. It was shown further that a significant amount of 2-deoxyglucose phosphate (30-60%) could be released from the glycogen fraction after it had been prepared by standard methods. Proof that 2-deoxyglucose could form glycogen was obtained by demonstrating that glycogen which had been stripped of adsorbed phosphates by passage over a Dowex 1 column and exhaustive dialysis could be degraded quantitatively by amylo-1, -4, 1-6-glucosidase to a substance which could pass through an Amicon PM-10 filter membrane. Treatment of the glycogen with phosphorylase "a" released 78% of the label in the form of a compound which did not migrate with 2-deoxyglucose on thin layer chromatography, and which upon mild acid hydrolysis at 80°C migrated in the same spot as 2-deoxyglucose.

Studies in which the 2-deoxyglucose and 2-deoxyglucose-6-phosphate were removed from brain homogenates by pretreatment with ATP and hexokinase followed by Dowex 1 anion exchange resin have made it possible to detect the free 2-deoxyglucose which can be released from glycogen by the enzyme amyloglucosidase.

If the brains are obtained by the freeze blowing method about 2% of the radioactive material will be present as glycogen whereas if the brains are dissected out, the rapid phosphorolysis of glycogen which occurs postmortem reduces the 2-deoxyglucose counts found in glycogen to 0.7% of the total radioactivity present in the brain. The further metabolism of 2-deoxyglucose-6-phosphate to glycogen is of no significance to the validity of conclusions which can be drawn from the results obtained with the 2-deoxyglucose method as it was originally described since the ^{14}C label trapped at any step beyond the phosphorylation step by hexokinase will represent glucose which has been metabolized.

If an attempt is made to apply the 2-deoxyglucose method to problems which require that labeled tissues be rinsed in water-containing solvents, then much of the 2-deoxyglucose-6-phosphate will be lost, leaving some or most of the less soluble 2-deoxyglucose labeled glycogen. Thus the label no longer is directly related to cellular glucose metabolism, but is also related to the individual cell's ability to synthesize glycogen. Moreover, since brains which are to be used for histological examination are usually carefully dissected from the skull, the variable delay between death and freezing may be accompanied by unpredictable losses of glycogen since this macromolecule is rapidly phosphorylated after death.

Proposed Course.

The project has terminated this year. The results have been written and are being submitted for publication.

Significance to Biomedical Research and Program of Institute.

Energy metabolism of the central nervous system has been the dominant interest of this Section since its establishment in 1953 when the Intramural Research Program was first organized. The studies described in this project are continued investigations along paths opened by previous research of the Section. The metabolic effects of γ -hydroxybutyrate were discovered here, and the present studies are designed to uncover the mechanism of its actions and, because it is normally present in brain, to elucidate its normal functions. It has been implicated in control of dopaminergic functions, in seizure states, and in anesthesia. The clarification of its origin, disposition, and function should serve to enlarge our knowledge of normal and abnormal functions and biochemistry in the brain.

Similarly, the first evidence that ketone bodies may play a role in brain energy metabolism was discovered here in this Section. It was subsequently shown by others that ketone bodies may substitute for glucose as a substrate for cerebral oxidative metabolism. There is, however, evidence that prolonged ketotic states may alter brain function. Diabetic keto-acidosis, for example, produces coma. Ketogenic diets diminish epileptic seizures in juvenile

epilepsy. The present studies are directed at elucidating the mechanism of these effects and may lead to better understanding and treatment of epileptic states and some types of coma.

PUBLICATIONS:

- Kaufman, E.E., Relkin, N., and Nelson T.: Regulation and properties of an NADP^+ oxidoreductase which functions as a γ -hydroxybutyrate dehydrogenase. J. Neurochem. 40: 1639-1646, 1983.
- Crosby, G., Ito, M., Kaufman, E., Nelson, T., and Sokoloff, L.: Naloxone pretreatment alters the local cerebral metabolic effect of γ -hydroxybutyrate in rats. Brain Research, in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00882-16 LCM
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Regional Cerebral Circulation and Metabolism		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Louis Sokoloff, Chief, Laboratory of Cerebral Metabolism, DBBR, NIMH		
COOPERATING UNITS (if any) Lab. of Neurobiology, NIA NIH; Theoret. Stat. Mathematics Br., NIMH; Lab. of Clinical Science, NIMH; Lab. of Psychology & Psychopathol., NIMH; Biolog. Psychiatry Br., NIMH; Dept. Obstetrics & Gynecology, Univ. Florida, Gainesville; Cornell U., NYC.		
LAB/BRANCH Laboratory of Cerebral Metabolism, DBBR, NIMH		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland		
TOTAL MANYEARS: 14.0	PROFESSIONAL: 6.5	OTHER: 7.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin-top: 10px;"> A method has been developed for the quantitative determination of the rates of <u>local glucose consumption</u> in the discrete functional and structural components of the brain in conscious or anesthetized laboratory animals. The method is based on the use of [¹⁴C]deoxyglucose as a tracer for glucose flux through the hexokinase step. Local [¹⁴C]deoxyglucose-6-phosphate concentrations in the tissues of the CNS are measured by a quantitative autoradiographic method. Inasmuch as the autoradiographs of the relative rates of local glucose consumption can be used directly for mapping <u>metabolically</u>, and therefore functionally, linked structures in the CNS, the method is being used to study alterations in the <u>energy metabolism</u> of the discrete functional and structural components of the brain in a variety of physiological, pharmacological, and pathological states. </p>		

OTHER INVESTIGATORS:

Charles Kennedy	Guest Worker	LCM NIMH
Massako Kadekaro	Visiting Scientist	LCM NIMH
Carolyn B. Smith	Staff Fellow	LCM NIMH
Masanori Ito	Visiting Associate	LCM NIMH
Linda Porrino	Staff Fellow	LCM NIMH
Hiroki Namba	Visiting Fellow	LCM NIMH
Paul Gross	Visiting Fellow	LCM NIMH
Astrid Nehlig	Guest Worker	LCM NIMH
Giovanni Lucignani	Visiting Fellow	LCM NIMH

COOPERATING UNITS:

S. Rapoport	Laboratory of Neurobiology	NIA NIH
J. Saavedra	Laboratory of Clinical Science	LCS NIMH
C. Patlak	Theoretical Statistics and	B NIMH
K. Pettigrew	Mathematics Branch	B NIMH
R. Esposito	Lab. Psychology & Psychopathology	LPP NIMH
T. Seeger	Biological Psychiatry Branch	BPB NIMH
A. Pert	Biological Psychiatry Branch	BPB NIMH
R. Abrams, Dept. of Obstetrics & Gynecology, Univ. of Florida		
T. Duffy, Depts. Neurology & Biochem., Cornell Univ. Medical Coll., NYC		

Project Description:Methods employed and significant results obtained.

Previous work in this Laboratory led to the development of a method for the measurement of the rates of blood flow in the structural and functional units of brain in conscious laboratory animals. The method was based on the uptake of a radioactive, chemically inert gas into the tissues of the brain, and a unique quantitative autoradiographic technique was developed which made possible the measurement by densitometric procedures of the concentrations of the radioactive tracer in the individual structures of the brain down to a resolution of 0.2-0.5 millimeters. The key to the fine resolution of the method was the autoradiographic technique.

Although measurement of local cerebral blood flow is inherently interesting with respect to the physiology, pharmacology, and pathology of the circulatory system, it is of limited value in studies of cerebral functional and biochemical activity. The Laboratory, therefore, addressed itself to the development of a method to measure local cerebral energy metabolism with the same degree of structural resolution because energy metabolism could be expected to relate more closely to local cerebral functional activity. It was always anticipated that the quantitative autoradiographic technique designed for the blood flow method would also be at the heart of such a method. It was necessary, however, to choose an appropriately labeled precursor of cerebral energy metabolism. Oxygen could not be used because there are no suitable radioisotopes of oxygen. [^{14}C]Glucose also appeared to be unsuitable because glucose is too rapidly metabolized, and its radioactive products are too quickly removed from brain. It was, therefore, decided to use [^{14}C]deoxyglucose, an analogue of glucose which is handled qualitatively just like glucose by the transport system in the blood-brain barrier and by the initial enzyme, hexokinase, in the pathway of glucose metabolism. Once phosphorylated, however, the deoxyglucose is trapped, unlike glucose which is metabolized further to carbon dioxide and water. Quantitatively, however, deoxyglucose phosphorylation and glucose phosphorylation or utilization are different

inasmuch as the transport carrier and the enzyme discriminate kinetically between the two substrates. It appeared to be a simple matter to apply the autoradiographic technique to measure deoxyglucose phosphorylation, but to relate it to the steady state rate of glucose flux through the phosphorylation step, which is a measure of the rate of glucose consumption, required the solution of numerous theoretical and technical problems.

A theoretical model, which encompassed all that we knew about deoxyglucose and glucose transport between brain and blood and their metabolism in brain tissue, was constructed, and mathematical relationships to describe the model were developed. Experiments were done on one point or another to evaluate and, if necessary, to revise the model and the mathematical relationships to fit the model closer to the real situation.

It was clear from the model that to determine the rate of glucose consumption from the rate of [14 C]deoxyglucose phosphorylation would require the determination of the distribution volumes of deoxyglucose and glucose in the cerebral tissues and the hexokinase kinetic constants (V_{\max} and K_m) for both deoxyglucose and glucose. By appropriate mathematical manipulations, it was possible to segregate all these separate constants into a single "lumped constant" encompassing all of them. It was now necessary to determine only the single lumped constant rather than the six individual ones. Further mathematical analyses revealed the way to design an experiment to determine the "lumped constant". Another equation was developed from the model which showed that if the arterial concentration was maintained constant for a sufficient length of time, e.g., at least 20 minutes, then the ratio of the cerebral extractions of deoxyglucose and glucose would reach a constant level equal to the lumped constant. With the help of the Theoretical Statistics and Mathematics Branch, it was found possible to derive from the analyses of plasma disappearance curves of deoxyglucose an intravenous infusion schedule which results in a constant arterial deoxyglucose concentration for up to 45 minutes or longer. Surgical procedures were developed in the rat, monkey, and cat to sample arterial and cerebral venous blood from which the extraction ratios are determined. The lumped constant has been fully determined in the conscious and anesthetized rat; its value is 0.48, and it is unchanged in a variety of physiological and pharmacological states. The lumped constant has recently been determined in the monkey and the cat; the values have been found to be 0.344 and 0.41, respectively. In collaboration with T. Duffy of the Department of Neurology, Cornell University, the lumped constant has been measured in the dog and found to be 0.56. In a collaboration with Dr. Robert Abrams at the University of Florida it has been measured in fetal and neonatal sheep. The values in pre- and postnatal life were virtually identical: 0.40.

All the theoretical and technical problems were solved, and the method has now been completely operative for the last six years. An equation has been derived which relates the rate of glucose consumption to measurable variables and allows the calculation of glucose consumption in the discrete structural and functional units of the brain. The equation prescribes the procedure to be used and the variables to be measured. An intravenous pulse of [14 C]deoxyglucose is injected, and arterial plasma concentrations of [14 C]deoxyglucose and glucose are measured from the time of injection until 30-45 minutes when the animal is decapitated, and the head frozen. Sections of brain are prepared from which local cerebral tissue [14 C]deoxyglucose concentrations are determined by the quantitative autoradiographic technique. From these measured variables and the lumped

constant, local cerebral glucose utilization is calculated by the equation. The procedure for calculation has been programmed, and all the calculations are carried out by a computer.

The regional localization obtained with the [^{14}C]deoxyglucose method is achieved by the use of quantitative autoradiography. The autoradiographs provide pictorial representations of the relative, not the actual, rates of glucose utilization in all structures of the brain. They are ordinarily subjected to manual densitometric analysis from which local ^{14}C concentrations are derived and used in the operational equation to compute the actual rates of local glucose utilization. We have recently developed a computerized image-processing system to analyze and transform the autoradiographs into color-coded pictorial maps of the actual rates of glucose utilization throughout the entire CNS. The autoradiographs are scanned automatically by a computer-controlled scanning microdensitometer which measures the optical density of each spot, 25-100 μm , in the autoradiograph. These optical densities are stored in a computer, converted to tissue ^{14}C concentrations on the basis of the optical densities of calibrated ^{14}C plastic standards, and then converted by the computer to actual local rates of glucose utilization by solution of the operational equation. Colors are assigned to narrow ranges of the rates of glucose utilization, and the autoradiographs are then displayed on a color monitor in color along with a calibrated color scale for identifying the rate of glucose utilization in each spot of the autoradiograph from its color. These pictures are, therefore, complete color-coded maps of the actual rates of local glucose utilization precisely localized in each 25-100 μm region of the CNS. Work is in progress to develop computerized techniques to reconstruct color maps of the entire brain three-dimensionally from the digitized autoradiographs. This would make it possible to enter into the computer all the data for the entire brain, sectioned in one plane, for example, serial coronal sections. The computer could then rotate the brain and section it from any direction, thus providing horizontal, or parasagittal sections as well. This would facilitate the identification of areas of altered cerebral metabolism in their three dimensions. The computer programming is complex, but significant progress has been made. Key elements of an algorithm to rotate images of sections, one at a time, and line them up with preceding sections have been developed. This work was temporarily interrupted by personnel changes but is now being resumed.

The deoxyglucose method was originally developed for use with ^{14}C quantitative autoradiography. ^{14}C was chosen because of the availability of ^{14}C X-ray film sensitive to its β -radiations. The resolution of the method with ^{14}C is 50-100 μm . Because ^3H has β -radiation of considerably less energy, it is possible to achieve finer resolution, e.g., 10 μm , with [^3H]deoxyglucose, provided, ^3H -sensitive film were available. X-ray film (LKB [^3H] Ultrofilm) sensitive to ^3H has now become available, and the necessary experiments to adapt the deoxyglucose method for use with ^3H have been carried out. A set of [^3H]methylmethacrylate standards has been calibrated to quantify the autoradiography, and the method has been applied to a series of normal rats to compare the results with those obtained with the [^{14}C]deoxyglucose. The autoradiographs with [^3H]deoxyglucose show finer resolution but much of the finer detail proves to be the result of differential absorption of the weaker β radiation from tritium as a result of the inhomogeneity of brain with respect to its fat content (myelin). It appears that the limiting factor in spatial resolution of the deoxyglucose method as currently carried out may be due to diffusion of the water soluble radioactive molecules during

processing of the tissues. Nevertheless the employment of tritiated deoxyglucose reveals much detail not seen when [^{14}C]deoxyglucose is employed as the tracer. Layers of the hippocampus and superior colliculus are clearly differentiated. Even single stellate-shaped cells of the anterior horns of the spinal cord are delineated. The studies represent a significant advance in the improvement of the resolution of the [^{14}C]deoxyglucose method and were recently published.

The deoxyglucose method was originally developed for use in animals in relatively normal physiological states. It was anticipated that in pathological situations the lumped constant and rate constants determined in normal animals would not be applicable in pathological states. Experience with the method has confirmed that in severe hypoglycemia and in hyperglycemia these constants do indeed change. In preceding years the lumped constant was determined at several different levels of hypoglycemia and hyperglycemia, and the rate constants at various blood glucose levels, extending to severe hyperglycemia, were estimated. The results of this initial series of studies revealed the rise in the value of one of the rate constants (k_2 , the rate constant for deoxyglucose's transfer from the exchangeable pool to the plasma) at 450 mg/100 ml of glucose and a subsequent decline at 550 mg/100 ml. This is contrary to the serial decline in the value for k_1 , the rate constant for the transfer of deoxyglucose from plasma to the exchangeable pool. Because of the unexpected difference in the direction of change in these two rate constants with increasing plasma glucose levels, it was decided to seek experimental confirmation by enlarging the number of these difficult experiments. Therefore, Dr. Astrid Nehlig and Dr. Giovanni Lucignani joined the project and recently have completed an additional block of experiments doubling the original series. The data are currently being analyzed by Dr. Clifford Patlak and Dr. Karen Pettigrew. When these studies are completed, not only will it be possible to study the effects of extreme changes in blood glucose level on local cerebral glucose utilization, but also to apply the method to pharmacological and physiological states that markedly alter the blood glucose level, e.g., epinephrine infusions, stress, diabetic acidosis and coma, etc.

Previous studies in this Laboratory demonstrated that dopamine agonists, such as d-amphetamine or apomorphine, activated glucose utilization in all the components of the dopaminergic pathways of the extrapyramidal motor system. These effects were blocked by dopamine antagonists, such as haloperidol, which alone produced opposite effects. A surprising finding was the absence of any effects on the dopaminergic mesolimbic system, which has been hypothesized to be overactive in amphetamine psychosis and, perhaps, also in schizophrenia. The previous studies were carried out acutely following single intravenous doses of the drugs. Amphetamine-psychosis characteristically occurs, however, after chronic use of amphetamine. The previous studies with d-amphetamine, have, therefore, been repeated with various modes of administration of the drug. They have confirmed that acute doses of d-amphetamine do not affect the mesolimbic system, but following continuous administration by osmotic pumps, glucose utilization is markedly activated in regions of the nucleus accumbens. These results lend support to the hypothesis that chronic amphetamine administration results in increased activity in the nucleus accumbens which may underlie the development of amphetamine psychosis.

The previous studies of acute amphetamine administration were all carried out with larger doses that elicited stereotypic behavior and showed only metabolic activation of the extrapyramidal motor pathways. Dr. Linda Porrino recently

examined the effects of lower acute doses of amphetamine, sufficient to produce clear behavioral effects manifested in enhanced locomotor performance but no stereotypy. Surprisingly, she found biphasic effects of d-amphetamine. At low doses between 0.1 and 1.0 mg/kg, locomotor activity was enhanced, and glucose utilization was activated in the nucleus accumbens. With increasing dosage up to 5.0 mg/kg the locomotor behavioral changes disappeared, and so also did the effect in the nucleus accumbens. In contrast, stereotypic behavior became manifested, and the extrapyramidal system was metabolically activated. These results not only shed light on the central nervous pharmacological effects of amphetamine but also provide good evidence of specific relationships between the problems of behavior and the distribution of changes in local glucose utilization in the brain.

In previous years Dr. Carolyn B. Smith applied the deoxyglucose method to the problem of normal aging in rats. She found selective decreases in local cerebral glucose utilization with age with the most profound effects in all the components of the primary auditory and visual pathways. These were effects similar to those seen following sensory deprivation of these systems. These results raised the question of whether or not some of the central nervous consequences of normal aging might not be due to sensory deprivation due to sense-organ degenerative changes inasmuch as there is known to be some retinal and inner ear degenerative change with age. Decreases in glucose utilization were also seen in the basal ganglia. These are structures which are part of the nigrostriatal dopamine system, and glucose utilization in these structures is normally activated by dopamine-agonists and depressed by dopamine antagonists. In order to determine whether the decreases with aging were due to loss of functional dopamine receptors, she initiated studies of the effects of normal aging on the metabolic responsiveness of these structures to the administration of the specific dopamine agonist, apomorphine. The results indicate that there is a loss of responsiveness with age; in the oldest age group, 24 months and older, there is essentially no metabolic response to apomorphine. These results suggest that a loss of functional dopamine receptors in the nigrostriatal system occurs with age, a change that may help to explain senile parkinsonism.

Drs. Massako Kadekaro and Paul Gross, in collaboration with Dr. Juan Saavedra of the Laboratory of Clinical Science, have been applying the deoxyglucose method to studies of the Brattleboro rat. This is a genetic strain of the Long-Evans rat which suffers from a defect in vasopressin synthesis and exhibits a characteristic diabetes insipidus. Despite the failure in vasopressin synthesis, the neurohypophysis shows marked increases in glucose utilization. It is as though the gland works harder because it cannot release vasopressin. Parenteral vasopressin administration in doses adequate to control the diabetes insipidus does not reverse this metabolic activation. Other components of the hypothalamico-hypophyseal tract, e.g., supraoptic and paraventricular nuclei, are not affected. The only other structure which shows an increased glucose utilization is the subfornical organ. This structure is known to mediate drinking in response to high plasma levels of angiotensin II, and the Brattleboro rat exhibits elevated drinking behavior and has high plasma concentrations of angiotensin II. Recently completed studies have demonstrated that angiotensin infusion does, in fact, stimulate metabolism in the subfornical organ and the posterior pituitary, evidence that high angiotensin levels contribute to the activation of the neurohypophyseal system in the Brattleboro rat.

Drs. Linda Forrino and Hiroki Namba have been using the deoxyglucose method to identify neuronal circuits upon which the female sex hormones, estrogen and progesterone, act to regulate sexual behavior in the female rat. In ovariectomized rats estrogen alone stimulates glucose utilization in the mid and posterior portions of the hypothalamus. Progesterone alone has no effects but when administered following pretreatment with estrogen depresses activity in the anterior-preoptic hypothalamic area. These results demonstrate an anatomical separation of the effects of female gonadal steroids in the hypothalamus and may reflect the effects of these hormones on female sexual behavior.

An earlier study in this laboratory was the local metabolic responses in brain throughout the diurnal cycle, in particular, the response of the supra-chiasmatic nucleus (Schwartz, W.J. and Gainer, H., *Science*, 197: 1089-1091, 1977; Schwartz, W.J. et al., *J. Comp. Neurol.*, 189: 157-167). Dr. Masanori Ito employed the [^{14}C]deoxyglucose method in an effort to learn whether the metabolic rate of the pineal gland of the monkey is altered in the course of the day-night cycle. Rates were measured in this tissue in awake animals entrained in the normal day-night cycle; four in darkness but awake at night, and three (with eyes occluded) awake in the daytime. Pineal metabolic rates in the nocturnal animals were 80-111% above those studied during the day. Short-term visual deprivation during the day was without effect on pineal glucose utilization. The findings suggest that accompanying the known role of the pineal in its formation and secretion of melatonin, there is a diurnal rhythm in its energy metabolism.

In an extension of the Laboratory's program in developmental neurochemistry Dr. Charles Kennedy has collaborated with Dr. Robert Abrams of the University of Florida in applying the [^{14}C]deoxyglucose method to fetal life. The studies were conducted in sheep, a species suited to the experiments because of its docility, the size of the fetus and its tolerance of the surgical preparation without inducing an abortion. Experiments were first undertaken to determine the lumped constant, a species specific constant in the operational equation of the [^{14}C]deoxyglucose method. The mean value in five fetuses was 0.416 (SEM \pm 0.01). This was not significantly different from 0.382 (SEM \pm 0.01) in four neonates. Local cerebral glucose utilization was then determined in representative structures of the brain at various ages from mid gestation until term. For comparison measurements were also made in three neonates. Rates of glucose utilization were generally low and homogeneous in mid gestation. The exceptions were elevated rates in the nuclei of the auditory and vestibular pathways and hippocampus. In the last seven weeks of gestation rates in virtually all structures rose three-fold. After birth there was a further average increase of 50% above those of the term fetus. The study showed that there was a rise in energy metabolism during prenatal maturation which occurred both in structures receiving sensory stimulation and in those which were not involved in any behavioral equivalent of functional maturation.

The effect of two drugs, both having selective actions on brain function, namely morphine and diazepam, have been examined for their effects on local cerebral metabolism. Dr. Masanori Ito conducted these studies. Morphine had been found in earlier experiments in this laboratory to have a generally depressant action on glucose utilization throughout the brain. However, the effect could be explained in part by the fact that the animals also developed respiratory depression resulting in an elevation of carbon dioxide tension in the arterial blood. Because carbon dioxide is known to reduce cerebral glucose

utilization, the direct action of morphine was left in question. Dr. Ito administered morphine in lower doses which were free of any depressant action on respiration and yet were clearly sufficient to alter behavior in specific test procedures. He was able to show an effect of morphine which selectively increased glucose utilization in the substantia nigra reticulata and in the mamillary nuclei. In the same experiments the medial portion of the lateral habenula was reduced in its rate. The results may be of importance to the understanding of morphine's antinocioceptive action in the light of other studies in which nociception has been found to involve a nigro-thalamic pathway.

Diazepam was found to result in a widespread lowering of cerebral metabolic rate in a dose-dependent manner. The lowest doses employed (0.25 mg/kg) were found to produce selective decreases in cerebral cortex and limbic structure. These findings may bear upon diazepam's anxiolytic action.

Caffeine, an inhibitor of cAMP phosphodiesterase and a blocker of adenosine receptors, produces a variety of specific behavioral and mental responses suggesting its action may be highly localized in its alteration of brain function. Dr. Astrid Nehlig, Dr. Giovanni Lucignani, Dr. Massako Kadekaro, and Dr. Linda Porrino have evaluated the effect of caffeine on local cerebral metabolism with the deoxyglucose method in albino rats. They have administered the drug intravenously in a range of doses, the highest of which (10 mg/kg) produced overt behavioral changes. At this dose local metabolic increases in brain structures involved with motor control were consistently found. Also the highest dose resulted in increases in structures concerned with endocrine function, in particular the paraventricular nucleus, the arcuate nucleus, and median eminence. Also significantly affected were the ventral tegmental areas, anterior cingulate cortex, medial prefrontal cortex, septum and raphe nuclei. Thus, the behavioral effects of caffeine are accompanied by metabolic rises in structures known to be involved in motor and endocrine function. Other structures less well defined in the literature for their role in behavioral change, which were also found to have elevated rate, may play a role in the alertness and general mental facilitation which results from the administration of caffeine.

Dr. Linda Porrino in collaboration with Dr. Ralph Esposito of the Laboratory of Psychology and Psychopathology and Drs. Thomas Seeger and Agu Pert of the Biological Psychiatry Branch has been applying the deoxyglucose method to the study of freely moving rats working for rewarding brain stimulation to the ventral tegmental area. The self-stimulation phenomenon is recognized as a model of goal-oriented appetitive behavior. Results revealed a selective pattern of metabolic activation in the terminal fields of the ventral tegmental area including the medial prefrontal cortex, central and basolateral amygdala, nucleus accumbens, dorsal raphe, parabrachial area and the locus coeruleus. Significant increases in glucose utilization were seen as well in various sensory and motor structures involved in the performance of the lever-pressing task itself. These results show that self-stimulation behavior involves the activation of a selective, consistent and discretely organized neural system with representation at all levels of the neuraxis. In another experiment, comparing self-stimulation (contingent) to experimenter-administered stimulation to the VTA (non-contingent) has shown that although the pattern of metabolic activity near the stimulation site was similar, consistent changes in glucose utilization were not found when the electrical stimulation was non-contingently administered. These data indicate the changes in glucose utilization seen in self-stimulation are specific

to the performance of an appetitive, goal-oriented operant task. Work in progress is comparing the patterns of glucose utilization which result from self-stimulation to different brain sites in order to define areas in the brain in which reward is coded.

SIGNIFICANCE TO BIOMEDICAL RESEARCH AND PROGRAM OF INSTITUTE.

The deoxyglucose method has made it possible for the first time to measure the rates of glucose utilization simultaneously in all functional and structural components of the central nervous system of conscious, behaving animals and now also in man. Because the method was developed in our Laboratory, it has been our responsibility to survey its applicability to the various types of conditions in which it might be applicable. The program has, therefore, been somewhat heterogeneous covering a wide range of physiological, pharmacological, pathological, and altered behavioral states. The method and its wide-ranging usefulness has now been more or less established, and it is used extensively throughout the world in neuroanatomical, neurophysiological, neuropharmacological, psychiatric, neurological, and neurosurgical research, and its wide acceptance is directly related to the results of studies in this project.

FUTURE COURSE OF PROJECT.

The applications of the deoxyglucose method to studies of sexual behavior and the influence of sex steroids on local cerebral glucose utilization will be continued and extended to the effects of these hormones when administered in the critical period for determination of future sexual behavior just after birth. Studies on the effects of thyroid dysfunction on local cerebral glucose utilization will be completed. The studies of aging and the responsiveness of dopamine-receptive structures to dopamine-agonists will also be completed, but studies of aging, particularly the effects of sensory isolation, will be studied further. Studies of sleep will be continued with particular efforts to measure local cerebral glucose utilization during REM sleep. The studies on glucose utilization in the hypothalamico-hypophyseal system of the Brattleboro rat will also be continued with the goal of defining the mechanism of the metabolic activation in the neurohypophysis of the Brattleboro rat.

Publications:

McCulloch, J., Savaki, H.E., McCulloch, M.C., Jehle, J., and Sokoloff, L.: The distribution of alterations in energy metabolism in the rat brain produced by apomorphine. Brain Research 243: 67-80, 1982.

McCulloch, J., Savaki, H.E., and Sokoloff, L.: Distribution of effects of haloperidol on energy metabolism in the rat brain. Brain Research 243: 81-90, 1982.

Buchsbaum, M.S., Ingvar, D.H., Kessler, R., Waters, R.N., Cappelletti, J., van Kammen, D.P., King, A.C., Johnson, J.L., Manning, R.G., Flynn, R.W., Mann, L.S., Bunney, W.E., Jr., and Sokoloff, L.: Cerebral glucography with positron tomography. Arch. Gen. Psychiatry 39: 251-259, 1982.

- Kennedy, C.: Energy metabolism of the brain in perinatal brain insult. Mead Johnson Perinatal and Developmental Medicine Symposium, Monograph #17. Evansville, Ind., Mead Johnson and Co., 1981, pp. 30-42.
- Kennedy, C., Sakurada, O., Shinohara, M., Miyaoka, M.: Local cerebral glucose utilization in the newborn macaque monkey. Annals of Neurol. 12: 333-340, 1982.
- Sokoloff, L.: New techniques in the study of local brain activity in animal and man. In Buijs, R.M., Pévet, P., and Swaab, D.F. (Eds.): Chemical Transmission in the Brain, Vol. 55 of Progress in Brain Research. Amsterdam, Elsevier Biomedical Press, 1982, pp. 331-347.
- Crosby, G., Crane, A.M., Jehle, J., and Sokoloff, L.: The local metabolic effects of somatosensory stimulation in the central nervous system of rats given pentobarbital or nitrous oxide. Anesthesiology 58: 38-43, 1983.
- Orzi, F., Dow-Edwards, D., Jehle, J., Kennedy, C., and Sokoloff, L.: Comparative effects of acute and chronic administration of amphetamine on local cerebral glucose utilization in the conscious rat. J. Cerebral Blood Flow Metab. 3: 154-160, 1983.
- Orzi, F., Kennedy, C., Jehle, J., and Sokoloff, L.: Measurement of local cerebral glucose utilization with 2-[³H]deoxyglucose in the rat. J. Cerebral Blood Flow Metab. 3(Suppl. 1): S77-S78, 1983.
- Sokoloff, L.: Energy metabolism and hyperexcitability: introduction. In Jasper, H.H. and van Gelder, N.M. (Eds.): Basic Mechanisms of Neuronal Hyperexcitability. New York, Alan R. Liss, 1983, pp. 391-398.
- Sokoloff, L.: Measurement of local glucose utilization in the central nervous system and its relationship to local functional activity. In Lajtha, A. (Ed.): Handbook of Neurochemistry, Vol. 3. New York, Plenum Publishing Corp., 1983, pp. 225-257.
- Porrino, L.J., Lucignani, G., Dow-Edwards, D., and Sokoloff, L.: Different anatomical substrates for amphetamine-induced stereotypy and locomotion demonstrated by measurements of local rates of cerebral glucose utilization. J. Cerebral Blood Flow Metab. 3(Suppl. 1): S210-S211, 1983.
- Schuijer, F., Wilms, U., Orzi, F., and Sokoloff, L.: Brain edema and mortality after cerebral ischemia in the gerbil: effect of 2-deoxyglucose. J. Cerebral Blood Flow Metab. 3(Suppl. 1): S339-S340, 1983.
- Kuschinsky, W., Haller, C., Suda, S., and Sokoloff, L.: The effect of gamma-hydroxybutyrate on the relationship between local cerebral glucose utilization and local cerebral blood flow and on pial artery reactivity to changes in extravascular pH. J. Cerebral Blood Flow Metab. 3(Suppl. 1): S570-S571, 1983.
- Ito, M., Suda, S., Namba, H., Sokoloff, L., and Kennedy, C.: Effects of acute morphine administration on local cerebral glucose utilization in the rat. J. Cerebral Blood Flow Metab. 3(Suppl. 1): S574-S575, 1983.

- Kennedy, C.: Changes in glucose utilization in relation to activity in the central nervous system. In Jasper, H.H. and van Gelder, N.M. (Eds.): Basic Mechanisms of Neuronal Hyperexcitability. New York, Alan R. Liss, 1983, pp. 399-421.
- Yarowsky, P., Kadekaro, M., and Sokoloff, L.: Frequency-dependent activation of glucose utilization in the superior cervical ganglion by electrical stimulation of cervical sympathetic trunk. Proc. Natl. Acad. Sci. USA 80: 4179-4183, 1983.
- Smith, C.B.: Localization of activity-associated changes in metabolism of the central nervous system with the deoxyglucose method: prospects for cellular resolution. In Barker, J.L. and McKelvey, J.F. (Eds.) Methods in Cellular Neurobiology, Vol. 1. New York, John Wiley & Sons (in press) 1983.
- Kadekaro, M., Gross, P.M., Sokoloff, L., Holcomb, H.H., and Saavedra, J.M.: Elevated glucose utilization in subfornical organ and pituitary neural lobe of the Brattleboro rat. Brain Research (in press) 1983.
- Kadekaro, M., Savaki, H.E., Kutyna, F.A., Davidsen, L., and Sokoloff, L.: Metabolic mapping in the sympathetic ganglia and brain of the spontaneously hypertensive rat. J. Cereb. Blood Flow Metab. (in press) 1983.
- Sokoloff, L.: Measurement of local glucose utilization and its use in localization of functional activity in the central nervous system of animals and man. Recent Progress in Hormone Research, Vol. 39. New York, Academic Press, (in press) 1983.
- Sokoloff, L.: Regional brain metabolism: its measurement and use to map cerebral functional activity. In Proceedings of Bicentennial Academic Convocation, Harvard Medical School, October, 11-14, 1982 (in press) 1983
- Sokoloff, L.: Modeling metabolic processes in the brain in vivo. (NINCDS Conference on "Research Issues in Positron Emission Tomography"). Annals of Neurology (in press) 1983.
- Gooch, C., Rasband, W., and Sokoloff, L.: Application of computer-assisted image processing to autoradiographic methods for studying brain functions. Trends NeuroSci. (in press) 1983.
- Sokoloff, L.: Measurement of local cerebral glucose utilization: Its use in localizing normal and abnormal functional activities in the nervous system. Proceedings of 11th International Salzburg Conf. on Cerebrovascular Disease, (Sept. 22-25, 1982). Amsterdam, Excerpta Medica (in press) 1983.
- Sokoloff, L., Kennedy, C., and Smith, C.B.: The deoxyglucose method for the measurement of local glucose utilization and the metabolic mapping of functional neural pathways in the central nervous system. In Research Methods in Neurochemistry. (in press) 1983.
- Sokoloff, L., Kennedy, C., and Smith, C.B.: Metabolic mapping of functional activity in the central nervous system by measurement of local glucose utilization with radioactive deoxyglucose. In Handbook of Chemical Neuroanatomy, Vol. I. Amsterdam, Excerpta Medica (in press) 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00887-06 LCM
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Extended Visual System of the Macaque Monkey		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> Charles Kennedy, Guest Worker, LCM NIMH		
COOPERATING UNITS (if any) Laboratory of Neuropsychology, NIMH		
LAB/BRANCH Laboratory of Cerebral Metabolism, NIMH		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland		
TOTAL MANYEARS: <div style="text-align: center;">2.0</div>	PROFESSIONAL: <div style="text-align: center;">1.25</div>	OTHER: <div style="text-align: center;">0.75</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>deoxyglucose</u> method is being applied to the <u>monkey</u> to advance knowledge regarding the parts of the brain which are involved in the <u>processing of visual information</u>. By measuring rates of local cerebral glucose utilization in animals during their performance of tasks involving different types of visual stimuli we anticipate learning which parts of brain are involved in such functions as <u>discrimination</u>, <u>memory</u> and <u>motivation</u>. Also by studying animals at various ages, information will be obtained regarding the <u>maturation</u> of the visual processing system. </p>		

OTHER INVESTIGATORS:

Masanori Ito	Visiting Associate	LCM NIMH
Louis Sokoloff	Chief, Lab. of Cerebral Metab.	LCM NIMH

COOPERATING UNITS:

Kathleen Macko	Staff Fellow	LN NIMH
Mortimer Mishkin	Research Psychologist	LN NIMH

Project Description:

The goal of the collaborative effort initiated in 1978 was to map regions of monkey brain which were responsive to visual stimulation. The deoxyglucose method has been shown to be sensitive to even small differences in functional activity, and it was hoped that it might be possible to shed light on such complex aspects of visual function as discrimination, memory, or even the mechanism by which the brain assigns a value judgement on the character of visual stimulation and then initiates a response to it. The procedure followed is to prepare animals so that one hemisphere is completely deprived of visual input. One optic tract is sectioned as is the corpus callosum and forebrain commissures. Because the intact brain functions symmetrically and therefore has equal metabolic rates in all homologous structures, the finding of right-left differences in metabolic rates in the surgically prepared animals serves to identify the visually responsive regions. The experiments to date have demonstrated that these include the striate cortex and entire expanse of the circumstriate and inferior temporal cortex as far forward as the temporal pole.

The cortical areas related to vision were found to include the entire expanse of striate, prestriate, and inferior temporal cortex as far forward as the temporal pole, the posterior part of the inferior parietal lobule, and the prearcuate and inferior prefrontal cortex; subcortically, in addition to the dorsal lateral geniculate nucleus and superficial layers of the superior colliculus, the structures related to vision included large parts of the pulvinar, caudate, putamen, claustrum, and amygdala. These results, which are consonant with a model of visual function that postulates an occipito-temporo-prefrontal pathway for object vision and an occipito-parieto-prefrontal pathway for spatial vision, reveal the full extent of those pathways and localize their points of contact with limbic, striatal, and diencephalic structures.

A major project has been the delineation of the exact border between visual and non-visual cortex throughout this extended region. This has been facilitated by the computer-assisted image-processing system which makes possible the estimation of average values for histologically distinct cortical areas. The border separating visual from non-visual cortex has now been mapped in detail through the entire extent of striate cortex (Areas OB and OA) to the inferior convexity of the temporal lobe (Areas TEO and TE).

In other experiments the contribution of the commissural systems to these visually responsive cortical areas was determined. The commissural systems are those which transmit visual information from cortex across the mid-line to contralateral cortex. This was done by comparing average rates of glucose utilization in cortex in monkeys which had the optic tract alone sectioned with those which had had optic tract section plus commissural section. The results indicated that the commissural contribution is very largely due to a region designated TE in the anterior portion of the inferior temporal lobe.

These findings gave no explanation, however, for the results of anatomic investigations which indicate that there is a much wider distribution of commissural fiber projections to the visual cortical pathway. A possible explanation for the failure of a metabolic response to occur over the full extent of these projections is that the cortical cells must be continually activated by an intact retino-geniculato-cortical pathway.

To test this hypothesis monkeys were prepared by "blinding" the right hemisphere in a manner different from that employed previously, namely, optic tract section. Instead a longitudinal cut was made in the optic chiasm together with occlusion of the right eye. This preserved the anatomic continuity of right sided retinal innervation to the cortex while depriving it of stimulation. Through a comparison of glucose utilization in the right hemispheres of these animals and of those studied previously with right optic tract section, the functional effectiveness of commissural input with and without spontaneous retinal input could be evaluated. A finding of a higher level of metabolic activity throughout the inferior temporal cortex of the former would indicate that the commissural fibers do require the intact innervation from the retina in order to respond. A preliminary analysis shows that there is no consistent difference between the visual cortical metabolic rates of animals with the optic tract cut and those prepared with the chiasm cut and eye occluded. Thus any commissural contribution to vision in the prestriate-posterior temporal region from an intact retina appears not detectable in the limited number of studies analyzed to date. To explain this apparent metabolic inertia of an anatomically established pathway may require another experimental approach.

The functional development of the extended cortical visual system is being studied by preparing infant monkeys with unilateral optic tract section and forebrain commissurotomy. The deoxyglucose method is then employed in the manner described for the mature animals. From a knowledge of the maturational characteristic of visual function the age range for these experiments was chosen to be 1 day to 5 months. The results show that there are systematic, age-related changes in rates of glucose utilization in normal visual related cortex as well as right-left differences between intact and visually deprived cortex. In all cortical visual areas of the intact hemisphere glucose utilization was lowest in the youngest subjects and reaches a maximum at four months. A single study at six months of age is at adult levels suggesting that the lower, mature rate is achieved long before pubescence. As in adult monkeys, the intact hemisphere of infants shows a progressive decline in glucose utilization along the ventral cortical visual pathway. This gradient was seen in all animals but was shallowest in the two youngest. The deprived hemisphere had low rates of glucose utilization compared to the intact hemisphere at all ages. The differences were greatest in the primary visual cortex (area OC) and smallest in the most anterior temporal cortex (area TE). These differences varied systematically with the age of the animal with maturation being accompanied by a serial increase of the intact-deprived difference until four months of age when it reached a maximum. This age of the peaking of both the absolute rate in the intact cortex and the intact-deprived difference corresponds with the age when the animal attains the capacity for object recognition (Bachevalier and Mishkin, *Int. J. Psychophysiol.*, 1983).

In the experiments cited above many animals had been trained to respond to a specific visual stimulus with unimanual key-pressing to obtain a water reward.

Thus the same experiments which were used to map the extended visual system also provided information on the metabolic responses to motor activity. While the motor pathways of the brain have been identified by other techniques, and thus are known, these experiments served to delineate the specific subdivisions of many structures which selectively are activated in the unimanual key-pressing. They provided new information on somatotopic localization of arm-hand movements. This was particularly well-defined in the study of cerebellar cortex. A large part of Crus II of the ipsilateral cerebellar cortex was shown to be selectively responsive in the animals' performance of the task. Also participating, but with a lesser percent change, was the lateral portion of the vermis in lobules III-VI. Localized increments in the rate of glucose utilization were also noted in VL and VPL of the thalamus, part of the globus pallidus and discrete zones of cerebral cortex (S, S_{II}, M) and a part of the supplementary motor area. A noteworthy feature of this mapping study of motor activity is that a much greater metabolic increment was found in structure concerned with sensory monitoring of motor activity than in those related to the motor activity itself.

Significance to Biomedical Research and to Program of the Institute.

This project represents a collaborative effort between the Laboratory of Cerebral Metabolism and the Laboratory of Neuropsychology in which the specialized expertise of each Laboratory is brought to bear on the use of the deoxyglucose method to study higher nervous functions, in this case the higher level processing of visual information beyond the primary visual system. The advantage of this approach is the ability to examine all local regions of the brain simultaneously in unanesthetized animals. It is hoped that these studies will help to elucidate the regions of the brain involved in integrating sensory inputs and eliciting appropriate affective responses.

Proposed Course.

The analysis of data obtained in experiments already carried out will be continued. Yet to be undertaken are experiments with different types of visual stimulation and with visual stimulation associated with tasks requiring learning and memory.

The Laboratory of Neuropsychology is reporting on this project with Report No. Z01 MH 02033-05 LN, titled "Functional Mapping of Sensory Systems".

Publications:

Reported in Z01 MH 02033-06 LN.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00889-04 LCM
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) A Method for the Determination of Local Rates of Protein Synthesis in Brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> Carolyn Smith Staff Fellow LCM NIMH </div>		
COOPERATING UNITS (if any) CPB NIMH; LPP NIMH; LN NIMH; University of Michigan, Ann Arbor, MI; Washington University, St. Louis, MO; UCLA, Los Angeles, CA.		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Developmental Neurochemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">6.0</div>	PROFESSIONAL: <div style="text-align: center;">4.0</div>	OTHER: <div style="text-align: center;">2.0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A method is being developed for the estimation of local rates of <u>protein synthesis in brain</u> in vivo. The method is based on the use of <u>L-[1-¹⁴C]leucine</u> as a tracer for the incorporation of leucine into protein. Six kinetic models for the behavior of leucine on brain have been designed. By mathematical analysis of the <u>kinetics</u> of exchange of the amino acid between plasma and the tissue pool(s) and its incorporation into protein, equations have been derived for each model that define the rate of amino acid incorporation into protein in terms of the time course of plasma-specific activity, final tissue concentration of ¹⁴C, and experimentally determined kinetic constants. Tissue concentrations of ¹⁴C are determined by <u>quantitative autoradiography</u>. Experiments are being carried out to test the validity of the various models. </p> <p> The method is currently being applied to studies of aging, development, hypothyroidism, plasticity in the visual system, regeneration, and sleep. </p>		

OTHER INVESTIGATORS:

Louis Sokoloff	Chief, Lab. of Cerebral Metabolism	LCM NIMH
Charles Kennedy	Guest Worker	LCM NIMH
Hiroki Namba	Visiting Fellow	LCM NIMH
Masanori Ito	Visiting Associate	LCM NIMH

COOPERATING UNITS:

W. Mendelson	Research Psychiatrist	CPB NIMH
R. K. Nakamura	Senior Staff Fellow	LPP NIMH
Mortimer Mishkin	Acting Chief, Lab. of Neuropsychology	LN NIMH
B.W. Agranoff	University of Michigan, Ann Arbor, MI	
Robert Collins	Washington University, St. Louis, MO	
Michael Phelps	UCLA, Los Angeles, CA	

Project Description:

A method is being developed for the estimation of local rates of protein synthesis in brain in vivo. This method is similar to the [14 C]deoxyglucose method in that it is based on enzyme kinetic principles as applied to the measurement of reaction rates in vivo with labeled tracers as substrates. In order to measure the rate of the reaction, one must know the amount of labeled product formed in a given interval of time and the integrated specific activity of the precursor. In an in vivo experiment the precursor pool cannot be sampled and the specific activity determined directly. It is necessary, therefore, to design a model for the behavior of the precursor in vivo and by kinetic analysis of the model to derive a relationship between the entire history of the precursor specific activity in the plasma (which can be sampled and measured directly), the integrated specific activity of the precursor pool in the tissue, and the rate of the reaction. Six kinetic models with progressively increasing complexity to take as many of the processes and factors into account as possible have been developed. By mathematical analysis of the kinetics of exchange of the amino acid between plasma and tissue and its incorporation into protein an operational equation for each model has been derived. Studies are in progress to identify the simplest model that adequately describes the processes proceeding in vivo.

For all of the models we have chosen L-[1- 14 C]leucine as the radiolabeled tracer for this method because the $^{14}\text{CO}_2$ derived from its metabolism is rapidly diluted in the pool of CO_2 and cleared from the tissue. There are, therefore, no side-reactions with radioactive products other than the labeled protein. Our current and most comprehensive model (Model VI) for the behavior of leucine in brain includes an extracellular and two intracellular compartments. The intracellular compartments are the precursor pool for protein synthesis, consisting of the activated amino acid, and the metabolic pool, the receptacle for discharged amino acid and the products of protein degradation. On the basis of the results of biochemical studies reported in the literature we propose that the amino acid is activated at the cell membrane. Therefore, only amino acid derived from the extracellular pool feeds the precursor pool. This compartmentalization would preclude mixing of the leucine derived from protein degradation with the precursor amino acid pool for protein synthesis. In vivo, however, because the extracellular space is small, this mixing might occur outside the cell.

We are currently carrying out experiments to test this model. One of our experiments consists of the determination of the specific activity of brain leucyl-tRNA and plasma leucine in a rat in a steady state for both labeled and unlabeled

leucine in the plasma. If the leucine is reutilized, the specific activity of the leucyl-tRNA will never reach the specific activity of the plasma leucine because it will be constantly diluted by unlabeled leucine derived from protein degradation. We have worked out a schedule for the intravenous infusion of labeled leucine in order to achieve a constant plasma level. We have also developed a method for the extraction and determination of picomolar levels of leucyl-tRNA in brain. With the use of differential centrifugation, acid precipitation, and phenol extraction, yields of tRNA of 100-200 $\mu\text{g/g}$ brain can be achieved. Our best yields of leucine following deacylation of the tRNA at pH 10 are about 20 pmoles/g brain. Consequently we have had to develop an ultrasensitive method for determination of the level of leucine derived from leucyl t-RNA. The method (adapted from a published method of Airhart et al., 1974) is based on the formation of labeled fluorescent amino acid derivatives following reaction of the amino acid extract with labeled dansyl chloride. The dansylated amino acids are separated with HPLC, and the dansyl-leucine peak is collected and counted with double label liquid scintillation counting. With this method we can detect as little as 5 pmoles of leucine. The final results from this series of experiments will provide us with an answer to the question of reutilization of leucine derived from protein degradation and the half-life of the precursor pool. With these results we can test the validity of Model VI.

In another series of experiments we are determining the half-life of the precursor leucine pool in local brain regions. In these experiments animals are administered a programmed infusion of $L-[1-^{14}\text{C}]$ leucine designed to achieve and maintain a constant plasma level of $[^{14}\text{C}]$ leucine. We are determining the best-fitting rate constant for the turnover of the pool from the following measurements: the amount of label incorporated in protein following 5-15' infusions, the plasma leucine specific activity during the infusion, and the actual rate of synthesis as determined by pulse labeling experiments. Preliminary results indicate that the half-life of the pool in the frontal cortex in rat is 0.8 minutes.

In a third series of experiments we are determining the degree of dilution of the precursor pool by unlabeled leucine derived from protein degradation. In these experiments we are testing the effect of changing the plasma leucine concentration on the calculated rate of protein synthesis. If there is significant dilution of the precursor pool, the calculated rate of protein synthesis should vary directly with the plasma leucine concentration. The data obtained from this series of animals, i.e., the plasma level of leucine and $[^{14}\text{C}]$ leucine and the amount of $[^{14}\text{C}]$ leucine incorporated into protein, can be applied to a fitting routine in order to determine the best-fitting fractional dilution of the precursor pool.

The model that best fits the known biochemical behavior of leucine in brain is Model VI, a multicompartment model with a very complex operational equation. We have simplified this equation. We have established that we can easily remove all of the free leucine from tissue sections without affecting the concentration of $[^{14}\text{C}]$ protein in the tissue by formalin fixation and washing. This procedure simplifies our expression for the rate of protein synthesis such that the determination of the amount of label in each leucine pool is unnecessary. Thus the constants needed in the equation are: 1) the half-life of the precursor leucine pool and 2) the degree of dilution of that pool. As our results suggest that the half-life is small (less than 1 minute), it can be shown that by 60 minutes after a pulse of $[^{14}\text{C}]$ leucine the integrated plasma specific activity approximately

equals the precursor pool specific activity. If the dilution factor is significantly different from 1, we are assuming that it does not change with our experimental conditions. Therefore, we are carrying out some studies on protein synthesis in brain in which we can obtain reasonable minimal estimates of the rates of protein synthesis. The other general assumptions of the method are:

- 1) steady state for protein and amino acid metabolism;
- 2) no breakdown of labeled protein;
- 3) tracer kinetics;
- 4) complete loss of $^{14}\text{CO}_2$ from the oxidation of L-[1- ^{14}C]leucine.

We have derived an equation that defines the rate of leucine incorporation into protein in terms of the following measurable variables: the time course of plasma-specific activity and the final tissue concentration of ^{14}C . The equation defines the procedure to be used and the variables to be measured. A pulse of [^{14}C]leucine (100 $\mu\text{Ci/kg}$ body weight) is administered intravenously, and the arterial plasma concentrations of labeled and unlabeled leucine are monitored for the duration of the experimental period. At 60 minutes the animal is killed by an intravenous injection of pentothal. The brain is removed, frozen, sectioned and washed and local tissue concentrations of ^{14}C are determined by quantitative autoradiography. We have calibrated new [^{14}C]methylmethacrylate standards for quantitative autoradiography with 10, 20, and 30 μm sections of brain. These standards are in a lower range than those used in the deoxyglucose method and will be more suitable for leucine autoradiographs.

We have determined rates of protein synthesis in a number of brain regions. We find a wide range of values from 1.5 nmoles leucine/g of tissue/min in white matter to 20 nmoles/g/min in some hypothalamic nuclei (e.g., the supraoptic nuclei). In general, brain regions that are rich in nerve cell bodies, such as the pyramidal cell layer in the hippocampus, and cranial nerve nuclei such as the dorsal motor nucleus of the vagus, have high rates of protein synthesis as compared to either white matter or regions composed largely of nerve terminals, dendrites, synapses, and axons, such as the caudate nucleus, thalamus and cortex. The value that we have obtained for cortex (5.0 nmoles leucine/g/min) compares favorably with values obtained by Dunlop et al. (1975) of 4.7 nmoles valine/g/min with a completely different method that yields only average values for the brain as a whole.

We are also carrying out several studies on the effects of specific treatments or conditions on local rates of protein synthesis. The purpose of some of these studies is to test the sensitivity of the method to detect changes in local rates of protein synthesis as well as to determine the responsiveness of protein synthesis to altered physiological states or pathological conditions. Experiments done in collaboration with Dr. R. Collins have shown that chemically-induced focal seizures produce a reduction in protein synthesis while stimulating glucose utilization. Studies on the effect of injury to the hypoglossal nerve have been carried out in collaboration with Dr. B. Agranoff. These studies have shown that cutting the hypoglossal nerve on one side will result in an increase in protein synthesis in its nucleus. We have studied the time course of this effect. We have also examined the time course of the effect of nerve section on glucose utilization in the nucleus. Our results show that the earliest change occurs in the rate of glucose utilization within 24 hours after nerve section. The increase in protein synthesis occurs later on day 4. The magnitude of the effect on glucose utilization is larger (70-80% increase over control) than that on protein synthesis (20-30%). Both of these metabolic responses return to normal by day 35. A

functional connection between the nerve and tongue is restored by day 24. A manuscript of these results is in preparation.

A study on the effects of age on local rates of protein synthesis in rats is being completed and prepared for publication. Two groups of rats were studied: young adult, 4-6 months; and aged, 15-23 months. The results of this study show that there is a significant decrease of approximately 17 percent in the overall rate of protein synthesis in the brain as a whole in the aged rats as compared to that of the young adults. Of the 39 individual structures in which the rates of protein synthesis were determined, 15 show significant or near significant age-related reductions. Eight of these are involved in sensory functions, e.g., vision, audition, and olfaction. The remainder include components of the extrapyramidal motor system, the limbic system, and the locus coeruleus.

The age-dependent changes in protein synthesis found in this study are in some respects similar to the age-related changes found in local rates of glucose utilization in rats (Smith et al., 1980). Changes in protein synthesis are indicative of long-term effects in the nervous system whereas changes in energy metabolism may occur with either acute or chronic alterations. The decreases in both of these biochemical processes in the sensory systems may reflect degenerative changes in the peripheral sense organs and secondary transneuronal effects in the CNS. The decrease in protein synthesis in the substantia nigra may be related to the decrease in glucose utilization found in the caudate nucleus, its major projection site. The decreased rate of protein synthesis in the locus coeruleus, the locus of the cell bodies of origin of ascending catecholaminergic projections, is particularly interesting as it may reflect either a cell loss or a decrease in the extent of these projections. A manuscript of these results is in preparation.

A study of the effects of chronic hypothyroidism and cerebral protein synthesis in adult rats was carried out. Two groups of male rats were studied: 1) rats which were surgically thyroidectomized three months prior to the study and 2) sham-operated controls. Of the 51 brain regions examined there were significant decreases in the rates of protein synthesis in 13 structures of the hypothyroid animals. These structures included mainly components of the extrapyramidal motor system, nuclei of cranial nerves, and hypothalamic nuclei. There were no significant changes in visual or auditory pathways or in any region of the cortex. Chronic hypothyroidism, therefore, appears to decrease rates of protein synthesis in a few selected areas of brain. These results are of interest because altered thyroid function in adults has been shown to influence the activity of several brain enzymes, to alter neurotransmitter levels and to modify behavior. A manuscript of these results is in preparation.

In collaboration with the Unit on Sleep Studies, and the Laboratory of Neuropsychology, we are also studying the effects of slow wave sleep on local rates of protein synthesis in monkey. This study is designed to test a long held hypothesis that sleep is a physiologic period during which the brain tissue undertakes repair and remodeling. To date a limited number of studies have been successfully completed. The autoradiographs are being analyzed on the computer-assisted image-processing system with the goal of obtaining representative values for rates of protein synthesis in approximately 80 brain structures. As was the case in the now completed study of local cerebral glucose utilization in non-REM sleep in the monkey, these will include those regions of the basal

forebrain, hypothalamus, midbrain, and brain stem which have received particular attention as "centers" for sleep regulation.

The method is also being used to study maturation and plasticity of the visual system in the newborn monkey. Prolonged monocular deprivation at birth results in a broadening of the ocular dominance columns representing the intact eye at the expense of the columns of the deprived eye. Eventually most of the striate cortex may be incorporated into a monocular visual system serving only the undeprived eye. The process underlying this reorganization is unclear. There may be an accelerated growth of terminals from the functional columns into the adjacent columns or a reduced growth and consequent retraction of the terminals of the non-functional pathway or a combination of both. The perikarya of the terminals in the striate cortex are located in well-defined layers segregated for the right and left eyes in the lateral geniculate bodies. We applied the [14 C]leucine method to the study of protein synthesis in chronic and acute monocular visual deprivation in newborn monkeys. Chronic monocular deprivation resulted in decreased rates of protein synthesis in the laminae of the lateral geniculate nuclei innervated by the deprived eye whereas in geniculate laminae innervated by the functioning eye rates of protein synthesis were normal. Acute monocular deprivation produced no differential changes in rates of protein synthesis in any of the geniculate laminae. These results suggest that the underdevelopment of the deprived columns is the result of inadequate growth and/or maintenance of axon terminals with consequent default of synaptic connections to the normally maintained terminals of the functional pathway. We are repeating these early studies with our modified procedure and simplified equation in order to delineate more precisely the magnitude of the changes in protein synthesis and to compare the values obtained in these animals with the rates obtained in animals following other forms of deprivation.

Results of neurophysiological and anatomical experiments suggest that it is the competition for synapses in the striate cortex that is affected by monocular deprivation and which ultimately leads to the domination of the cortex by the input from the non-deprived eye. Our protein synthesis results are consistent with this hypothesis and they suggest that the rate of protein synthesis may be involved in this reorganization. Alternatively, the effects on protein synthesis may be a consequence of this reorganization rather than an influence on it. In order to examine this question we have studied the effects of protein synthesis on binocular deprivation, i.e., monkeys in which both eyes are sutured closed from birth until day 25, at which time the study is carried out. Results obtained on two pairs of monkeys suggest that protein synthesis is decreased throughout the laminar of the lateral geniculates and the striate cortex in the binocularly deprived animals as compared to the age-matched controls. These results would suggest that even without a change in competitive advantage in the striate cortex protein synthesis is affected by a change in visual input.

We are following up these studies on the monkey visual system by looking at the recovery from monocular deprivation in the lateral geniculates and the striate cortex. In a monkey in which we opened the deprived eye after 25 days and sutured the previously opened eye for an additional 25 days the [14 C]leucine autoradiographs showed a reversal in the layers of the lateral geniculates, i.e., the layers innervated by the initially opened eye had lower rates of protein synthesis than those innervated by the initially deprived eye. Furthermore, in the striate cortex, columns with alternating high and low rates of protein synthesis were evident.

The columns were perpendicular to the cortical surface with a periodicity of about 0.8 mm. They were particularly distinct in layers 2-4. The portion of the period with the higher rate of protein synthesis was slightly wider. These results show that in the newborn monkey the reorganization that occurs in response to chronic visual deprivation is reflected in changes in protein synthesis in the cells along the visual pathway. The reduction in protein synthesis in the dLGN may underlie an inadequate maintenance of terminals in the striate cortex with a consequent loss of the competition to afferents from the nondeprived eye. The results of this reverse suture experiment suggest that the reorganization of the visual cortex that takes place in response to this experimental procedure also involves changes in protein synthesis in cortical cells.

Other experiments in progress are designed to further examine the processes involved in the plasticity demonstrated by this system. In addition, we have studied several prepubescent monkeys to test whether or not this protein synthesis response occurs in monkeys with fully developed visual systems. In two monkeys: 1) one year old monocularly deprived for 3 months and 2) 18 month old monocularly deprived for 6 months we found no significant effect on the rates of protein synthesis in the laminae of the lateral geniculates. We know, however, that in these monkeys that some reorganization at the level of the striate cortex has taken place because in another 18 month old monkey, monocularly deprived for 6 months, our deoxyglucose autoradiograph showed some irregularities in the metabolic picture of the ocular dominance columns.

In collaboration with Dr. M. Phelps of the Division of Nuclear Medicine, UCLA, an organization that has a functional positron-emission tomographic laboratory, we are trying to adapt the method for use in man. The synthesis of [$1-^{11}\text{C}$]leucine and the purification of the L-isomer have been worked out. The rate constants for our Model V have been determined in adult monkeys. With the fit to this two compartment model it appears that in adult monkey the metabolic pool has an 11-minute half-life. This presents some difficulties for the human application because the half-life of ^{11}C is only 20 min. Studies are in progress to achieve conditions which will shorten the half-life of the metabolic pool without affecting the rate of protein synthesis.

Significance to Biomedical Research and Program of the Institute.

Protein synthesis is probably the most important biochemical process underlying the development, maturation, plasticity, maintenance, and long-term regulation of the nature and degree of functional activity of the nervous system. The structural, functional, and metabolic properties of the tissues largely reflect the role of structural and enzymatic proteins. Peptides that are considered to be neurotransmitters are in some, and possibly all, cases derived from the cleavage of large parent protein molecules. Many hormones within and outside the nervous system are proteins. It is, therefore, certain that changes in protein synthesis can and do alter function and that some mental and neurological dysfunctions reflect disturbances in this vital biochemical process. This research is directed at determining the rates of protein synthesis in specific regions of the central nervous system with an ultimate resolution down to the cellular level. This provides for the first time the opportunity to study at the individual structural or anatomical level the changes in protein synthesis that may be the causes, consequences, or correlates of normal conditions, such as maturation, plasticity, differentiation, sleep, learning and memory, behavioral patterns, etc.,

or pathological conditions, such as hormonal disorders, aging, regeneration in response to injury, convulsive disorders, coma, etc.

Proposed Course.

We are concentrating our efforts on completing experiments on the question of dilution of the precursor pool specific activity with leucine derived from protein degradation. In the same experiments we are determining the half-life of the precursor pool. The applications of the method which are currently in progress will be continued; these include studies of CNS regeneration, slow wave sleep, aging, cretinism, and plasticity in the monkey visual system.

Publications:

- Smith, C.B. and Sokoloff, L.: Age-related changes in local glucose utilization in the brain. In Hoyer, S. (Ed.): The Aging Brain. Exp. Brain Research, Suppl. 5. Berlin/Heidelberg, Springer-Verlag, 1982, pp. 76-85.
- Sokoloff, L. and Smith, C.: Biochemical principles for the measurement of metabolic rates in vivo. In Heiss, W.-D. and Phelps, M.E. (Eds.): Positron Emission Tomography of the Brain. New York, Springer-Verlag, 1983, pp. 2-18.
- Sokoloff, L. and Smith, C.B.: Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In Lambrecht, R.M. and Rescigno, A. (Eds.): Tracer Kinetics and Physiologic Modeling. New York, Springer-Verlag, 1983, pp. 202-234.
- Smith, C.B.: The influence of age on cerebral energy metabolism. In Energy Transduction and Neurotransmission, Joint ESN-WFN Symposium, Rome, September 20-21, 1982. (in press) 1983.
- Ingvar, M., Maeder, P., Sokoloff, L. and Smith, C.B.: The effects of aging on local rates of protein synthesis in rat brain. (Symposium "Effects of Aging on Regulation of Cerebral Blood Flow and Metabolism", San Remo, June 30-July 1, 1983). Basel, Karger, (in press) 1983.
- Sokoloff, L. and Smith, C.B.: Basic principles underlying radioisotopic methods for assay of biochemical processes in vivo. In The Metabolism of the Human Brain Studied with PET, (Nobel Conference VII, May 17-20, 1983). Raven Press Books, Ltd., London (in press) 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00900-27 LCM
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Studies on Myelin and Myelin Basic Protein		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Russell E. Martenson, Research Chemist, LCM, NIMH		
COOPERATING UNITS (if any) Neuropathology Dept., Univ. of Washington School of Med., Seattle, Wash. School of Chemistry, The Univ. of Sydney, New South Wales, Australia Biophysics Res. Div., Univ. of Michigan, Ann Arbor, Michigan		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Myelin Chemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.5	PROFESSIONAL: 2.5	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Several avenues of work are being pursued: 1) <u>specific cleavages</u> of the central nervous system myelin basic protein to obtain suitable fragments for biochemical and immunological studies, 2) nuclear magnetic resonance studies to help elucidate the detailed <u>conformation</u> of the protein, 3) immunological studies aimed at delineating <u>antigenic sites</u> of the protein, 4) studies aimed at defining the complete <u>amino acid sequence</u> of the protein from pig brain, and 5) investigations into the <u>catalytic activity</u> of the protein.		

Project Description:Others Involved in this Project:

G. E. Deibler	Chemist	NIMH, LCM
M. L. Pedersen	Biologist	NIMH, LCM

Objectives:

The major objective of the current work has been to elucidate the three-dimensional structure of the myelin basic protein (BP) in aqueous solution and when bound to phospholipids in an attempt to understand the function of the protein in the myelin sheath.

Methods Employed:

The work has involved nuclear magnetic resonance and immunological studies of the BP and specific fragments thereof from a number of species as well as the preparation of large quantities of the BP fragments useful in such studies.

Major Findings:

1. We have recently isolated and purified a number of peptic fragments of the BP of rabbit brain. In a collaborative study with Dr. Walter Moore at the University of Sydney, these fragments have been used in nuclear magnetic resonance (NMR) studies to help elucidate the detailed conformation of the protein. Some of these fragments, along with additional fragments and intact basic proteins of other species have been used to assign resonances of the different threonine, tyrosine, and histidine residues in the protein. Since fragments were chosen that contain either none or some of these residues in common, and since the positions of these residues in the amino acid sequences of the proteins are known, comparisons of the NMR spectra made the assignments possible. The particular geometry and environment of a residue in a protein influences the extent to which its resonance peak is shifted from its position as a free amino acid. The occurrence of several distinct resonances for threonine, tyrosine, and histidine residues in the NMR spectrum of the basic protein indicates that the various residues of a given type (e.g., the different threonines) are physicochemically non-equivalent. Thus, when the secondary structure of the protein changes from a largely irregular one in aqueous solution to a partially helical one upon binding to phospholipid micelles, it should be possible to use the specific residues as "reported" groups to determine which parts of the protein undergo conformational transitions.

2. We have recently examined the conformation of the peptide derived from the 168-residue BP by peptic cleavage 9 residues before and 10 residues after the triproline sequence, yielding peptide 88-109. NMR spectral studies provide evidence that the triproline sequence serves as a rigid spacer between a highly structured sequence on its N-terminal side and a highly flexible sequence on its C-terminal side, thus dispelling the popular myth that the triproline sequence promotes a "hairpin turn."

3. Work in this lab has also involved the preparation and identification of fragments produced from the rabbit BP by thrombin cleavage. Thrombin, which is specific for certain arginine residues in proteins, under appropriate conditions cleaves the BP of several species tested at a single site, Arg₉₅-Thr₉₆. This site is located immediately before the triproline sequence. Incubation of the BP with higher concentrations of thrombin for longer periods of time results in the generation of additional peptides. Unlike the peptic peptides, which result from cleavage between hydrophobic residues, most of the thrombin peptides result from cleavages in the hydrophilic regions of the protein. The latter peptides contain the more highly structured regions of the protein and should be especially useful in studying regions of the protein that are predicted to undergo coil → helix transitions and/or to coalesce to form a compact globular structure.

4. Another avenue of work has been a collaborative study with Dr. Ellsworth C. Alvord, Jr., at the University of Washington to define the antigenic sites in BP that react with monoclonal antibodies. This work has been made possible by the availability of a number of basic proteins of different species and a variety of BP fragments, particularly those generated by pepsin and thrombin. Of the 7 different monoclonal antibodies that have been found to react with BP, two from rat react with a site in the N-terminal 14 residues, one each from rat and mouse reacts with a site near the Phe-Phe sequence in the middle of the polypeptide chain, one from rat reacts with a sequence near the single tryptophan residue, and two from mouse react with a sequence in the C-terminal fourth of the molecule. No two antibodies directed to the same general region have identical specificities. Of particular interest are two antibodies whose complete epitope (sites against which the antibody is directed) appears to be made up of at least two regions separated from each other in the sequence. One of these consists of a major site near the central Phe-Phe sequence; the other consists of a major site near the tryptophan residue. These monoclonal antibodies are potential conformational probes, since they should enable us to determine where folding in the BP occurs such that regions far apart in the sequence lie close together in the three-dimensional structure of the protein. Also of interest are certain anomalies associated with the epitope near the N terminus of the protein that suggest the presence of both cis and trans peptide bonds before the proline at position 6 in the polypeptide chain.

5. The BP from pig brain has been used by several investigators for immunological and physicochemical studies because of its commercial availability. The original partial sequence data indicated that the protein is very similar to the bovine BP, with several possible amino acid substitutions of particular interest. We are presently carrying out sequence studies on several of the tryptic peptides in order to determine the complete amino acid sequence of the pig BP.

6. An aspect of the BP that we are currently investigating with Dr. Robert Zand at the University of Michigan is an apparent intrinsic esterase and alkaline phosphatase activity. We have found that diisopropylfluorophosphate, a specific active site inhibitor of serine esterases and proteinases, reacts at several loci in the BP. Presumably, these labeled sites are part of catalytically active regions.

Significance to Biomedical Research and the Program of the Institute:

Knowledge gained from the studies described above are essential to an understanding of how the myelin sheath is assembled and maintained and, in addition, will provide insight into the nature of some of the pathological processes involving loss of myelin, in particular, plaque formation in multiple sclerosis.

Proposed Course:

We plan to continue our studies on the chemistry of the myelin basic protein, with emphasis on studies that will define more precisely its three-dimensional structure in solution and its conformation in the myelin sheath. We wish to explore the nature of basic protein dimerization and the residues involved in the intermolecular contacts, since dimerization of the protein across the cytoplasmic surfaces of the myelin lamellae could be a mechanism for myelin compaction.

Publications:

- Martenson, R. E.: Posttranslational modifications of myelin basic protein. In Peeters, H. (Ed.): Protides of the Biological Fluids. New York, Pergamon Press, 103-106, 1982.
- Agrawal, H. C., Martenson, R. E., and Agrawal, D.: In vivo incorporation of [³²P]-orthophosphate into myelin basic protein of developing rabbit brain. Its location in Components 3 and 5 and in a new protein tentatively identified as basic protein Component 7. J. Neurochem. 39: 1755-1758, 1982.
- Martenson, R. E., Law, M. J., and Deibler, G. E.: Identification of multiple in vivo phosphorylation sites in rabbit myelin basic protein. J. Biol. Chem. 258: 930-937, 1983.
- Mendz, G. L., Moore, W. J., and Martenson, R. E.: NMR studies on myelin basic protein. VIII: Complete assignment of the threonine residues by proton NMR of proteins from five species. Biochimica et Biophysica Acta 742: 215-223, 1983.
- Martenson, R. E.: A general model of the P₂ protein of peripheral nervous system myelin based on secondary structure predictions, tertiary folding principles, and experimental observations. J. Neurochem. 40: 951-968, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00901-28 LCM
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunologic Reactivity of Myelin Basic Protein		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Marian W. Kies, Chief, Section on Myelin Chemistry, LCM, NIMH		
COOPERATING UNITS (if any) Neuropathology Department, University of Washington School of Medicine, Seattle, Wash.		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Myelin Chemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 1.0	OTHER: 2.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Passive transfer of experimental allergic encephalomyelitis (EAE) in Lewis rats has been used to study pathogenetic mechanisms of autoimmunity. BP-sensitized lymph node cells (LNC) transfer EAE to naive recipients in an enhanced manner if they are cultured with specific antigen (BP). Enhancement can also be achieved, without specific antigen, if BP-sensitized LNC are incubated with supernatants prepared from spleen cells incubated with Con A. These supernatants have been variable in their ability to enhance transfer with BP-sensitized LNC. In trying to prevent and explain the variability, we have discovered that a second subset of cells (not specifically sensitized to BP) provides the extra boost required to achieve more consistent results. The components of the system which results in the most effective transfers are (1) BP-sensitized LNC pre-incubated in medium, then incubated in SpC/Con A supernatants; and (2) CFA-sensitized SpC incubated with LPS. The two cell suspensions are injected separately into the recipient. Successful transfer of severe EAE is routinely obtained with this cell mixture.</u> </p>		

Project Description:Others Involved in this Project:

B. F. Driscoll Research Biologist NIMH, LCM

Objectives:

Use of cell transfer to study immune mechanisms in the induction of EAE; specifically, to define the conditions under which culture with Concanavalin A (Con A) and lipopolysaccharide (LPS) activates BP-sensitized spleen and LNC cells.

Methods Employed:

Lewis rats are sensitized with BP/CFA (specifically sensitized) or CFA (non-specifically sensitized). Lymph node cells (LNC) and spleen cells (Sp C) are removed from these sensitized animals and used for passive transfer of EAE after the various manipulations indicated. In all of the experiments described, the proper controls have been carried out, e.g., BP-sensitized LNC were incubated alone and shown to be negative for passive transfer.

Major Findings:

BP-sensitized spleen cells cultured with Con-A transfer EAE in an enhanced fashion while BP-sensitized LNC cultured with Con A do not. We reported last year that the ability of BP-sensitized LNC to transfer EAE is enhanced by culturing them in spleen cell supernatants. SpC/Con A Sup is prepared by incubating either naive or CFA sensitized spleen cells with Con A for 24-48 hr. The BP-sensitized LNC require preincubation in medium alone for 24-48 h before they are capable of responding to the SpC/Con A Sup factor. One of the best characterized of the soluble factors induced by Con A stimulation of spleen cells is IL-2 (T-cell growth factor). IL-2 has been partially characterized and is known to be extremely stable. Although IL-2 has been suggested as the critical element responsible for enhanced transfer when spleen cells are incubated with Con A, we believe that our results suggest a more complex mechanism: (1) IL-2 is stable to freezing, storage at 4°C, lyophilization, etc.; in contrast, the factor in SpC/Con A Sup which enhances transfer of EAE by LNC is very labile. We have never observed enhanced transfer with stored or frozen preparations. (2) Exposure of spleen cells to Con A for 4 hr induces IL-2 production (and proliferation) but does not enhance transfer of EAE. (3) LNC produce IL-2 (and proliferate) when stimulated directly by Con A but require a pre-incubation period with RPMI/FCS before responding to Con A with enhanced transfer. (4) Although the stimulation of BP-sensitized LNC by SpC/Con A Sup was observed repeatedly, it was an inconsistent phenomenon. This latter point suggests strongly that other factors are involved which are not being efficiently produced under our experimental conditions.

In attempting to account for the variable results of the activation of LNC by SpC/Con A Sup we have discovered that more consistent and effective activation can be achieved by a) adding LPS to the medium in which the LNC are preincubated; or b) incubating CFA/spleen cells independently with LPS and injecting them into

the recipient at the same time that the BP-sensitized LNCs are injected. In either case, the LNC require pre-incubation followed by incubation with SpC/Con A Sup. LPS, which is known to stimulate inflammation in vivo, is a lipopolysaccharide isolated from E. coli.

While we cannot provide a good hypothesis yet for the enhanced transfers we are observing, our experiments indicate 1) that inflammation is an important part of CNS lesion formation and 2) that more than one cell type is essential for the enhanced transfer phenomenon. LNC provide the BP-sensitized cells; the spleen cells which produce the SpC/Con A Sup and the Sp C which have been incubated independently with LPS do not have to be BP-sensitized.

One prediction from these experiments is that BP-sensitized cells maintained in vitro with IL-2 and/or BP will not transfer EAE if they are cloned and subsequently cultured as a single cell line.

Significance to Biomedical Research and Program of the Institute:

This work represents an important addition to the pathogenetic mechanisms which have been characterized in the experimental disease, allergic encephalomyelitis. In the human idiopathic conditions thought to be caused by autoimmune reactions, the mechanism of sensitization, the effect of lymphokines on the reticuloendothelial system, and the initiation of the lesion in the target organ are all completely unknown. It has been thought for many years that multiple sclerosis may be an autoimmune disease. More recently, several investigators have suggested that Alzheimer's disease may also result from an adverse autoimmune reaction. It is important to work out possible mechanisms for the initiation and formation of CNS lesions in these conditions in order to provide a theoretical basis for treatment and/or prevention.

Proposed Course:

The most important problem at present is the identification of the non-specifically activated cells which interact synergistically in vivo with the BP-sensitized cells. These cells are probably responsible for producing inflammation at the site of a CNS lesion initiated by the BP-specific cells. This will be pursued not only by in vitro manipulations of these cells but also by comparing the histologic lesions of animals receiving various cell types. We have partially purified IL-2 from spleen cell/Con A supernatants and will compare the effectiveness of the partially purified factor with that of the whole SpC/Con A supernatants. Indirect evidence indicates that IL-2 contributes to enhanced transfer of EAE but other factors may be equally important.

Publications:

Kies, M. W.: Myelin Basic Protein. Scand. J. Immunol. 15: 125-146, 1982.

Richert, J. R., Kies, M. W., and Alvord, E. C., Jr.: Adoptive Transfer of Experimental Allergic Encephalomyelitis: Inhibition by Agents that Elevate Intracellular Cyclic AMP Levels. Neurochem. Pathol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00902-18 LCM
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Induction and Prevention of Experimental Allergic Encephalomyelitis (EAE)		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Bernard F. Driscoll, Research Biologist, LCM, NIMH		
COOPERATING UNITS (if any) Neuropathology Department, University of Washington School of Medicine, Seattle, Wash.		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Myelin Chemistry		
INSTITUTE AND LOCATION NIMH, ADAMAH, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.0	PROFESSIONAL: 2.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p style="margin: 0;">In attempting to understand the various cellular events involved in the pathogenesis of EAE we have used the phenomenon of <u>enhanced transfer</u> to study mechanisms of <u>immunity</u> and <u>inflammation</u> in delayed-type hypersensitivity.</p> <p style="margin: 0;">A. Immunity:</p> <p style="margin: 0;">1) <u>Strain specificity</u> of EAE induction in Strain 2 and Strain 13 guinea pigs;</p> <p style="margin: 0;">2) <u>Soluble factors</u> capable of activating cells for enhanced transfer;</p> <p style="margin: 0;">3) <u>Suppression</u> of EAE.</p> <p style="margin: 0;">B. Inflammation:</p> <p style="margin: 0;">The role played by non-specific inflammatory cells in development of EAE.</p> <p style="margin: 0;">C. Chronic EAE:</p> <p style="margin: 0;">A study of the antigens and mechanisms responsible for demyelination in the CNS. This phase of disease follows lesion initiation by antigen-specific cells and lesion development by inflammatory cells.</p>		

Project Description:Others Involved in this Project:

M. W. Kies Chief, Section on Myelin Chemistry NIMH, LCM

Objectives:

Dissection of the various cellular events involved in the pathogenesis of EAE including antigen-specific immune responses and non-specific inflammatory responses; investigation of the antigen(s) responsible for, and the mechanism of, demyelination in chronic EAE.

Methods Employed:

Various populations of immune cells are removed from strain 13 guinea pigs previously sensitized with myelin basic protein (BP) in complete Freund's adjuvant (CFA). After appropriate in vitro culture, these cells are injected into naive recipients. The recipients are observed for clinical signs of EAE to determine whether the in vitro treatment of cells was effective in enhancing transfer of EAE. In order to study chronic EAE, recipients of suboptimal numbers of BP-sensitized cells are injected with guinea pig spinal cord in CFA.

Major Findings:

EAE is induced in susceptible animals by injection of purified BP in CFA. The subsequent immune response leading to the development of disease has been shown to be a T-cell mediated, delayed type hypersensitivity (DTH) reaction.

DTH reactions are complex, involving 1) interaction of various classes of antigen-specific lymphocytes and 2) the attraction of non-specific leukocytes by these specifically sensitized cells, when the latter are exposed to specific antigen whether it is injected into the skin or, in the case of EAE, is an intrinsic part of the central nervous system. Therefore, our research involves examination not only of mechanisms of differentiation of BP-immune lymphocytes but also the mechanisms involved in development of inflammation, which is probably the major cause of the lesions in the CNS and indirectly the cause for the various clinical signs of EAE.

Immunity to BP - Three separate types of experiments which are concerned with the stimulation, development and suppression of antigen-specific cells, have been undertaken.

1) Species and strain specificity of EAE induction: The cells responsible for inducing EAE are stimulated by specific determinants on the BP molecule. The site responsible for induction of EAE in strain 13 guinea pigs includes residues 113-121 which occur in the C-terminal peptic peptide, Res. 89-169. Since the site on the BP molecule that is encephalitogenic is genetically determined, the site responsible for disease induction varies from species to species. It now appears that the region of the molecule responsible for disease induction can vary even within a single species. We have demonstrated that

strain 2 guinea pigs and strain 13 guinea pigs respond to different sites on the BP molecule. The site responsible for EAE induction in strain 2 guinea pigs was demonstrated by culturing whole CNS-sensitized cells in the presence of purified fragments of BP and subsequently transferring these cells to naive recipients. In such cases strain 13 guinea pig peritoneal exudate cells (PEC) proliferate and transfer EAE when cultured with peptic fragment (89-169) but not with fragment (1-88). The opposite is true for strain 2 PEC -- fragment (1-88) is active and fragment (89-169) is inactive.

2) Soluble factors important in enhanced transfer: Once T cells are stimulated by specific determinants on the antigen, their development is controlled by various non-specific factors. Among the most important factors responsible for development of T cells is T cell-growth factor, now called interleukin 2 (IL-2). For efficient transfer of EAE with strain 13 guinea pig lymph node cells (LNC), these cells must be cultured not only with specific antigen (BP) but also with allogeneic (strain 2) PEC. Culturing allogenic cells together induces a mixed leucocyte response (MLR) characterized by intense cellular proliferation accompanied by production and release of multiple factors. In an attempt to analyze the role of soluble factors in these cultures, we have used various culture supernatants in place of the allogeneic PEC in culture. After exposure to antigen alone, BP-LNC are incapable of transferring disease; after subsequent culture in supernatants rich in IL-2, these cells exhibit intense proliferation but still transfer disease poorly. However, after culture in MLR supernatants, the LNC can transfer severe EAE while undergoing little or no proliferation. MLR supernatants do contain low levels of IL-2 but it is apparent that this factor alone is insufficient to induce the cells to transfer disease. Some other, as yet undetermined factor(s) present in the MLR supernatants are responsible for activating the cells for disease transfer. The cells producing or responding to the factor(s) have not been characterized.

3) Suppression - a phenomenon of suppressor cells? One segment of the immune reaction works in a negative fashion and dampens or suppresses immune reactivity. T-suppressor cells have been identified in many in vitro systems. We have reported a method for inducing potent suppression of EAE which involves injecting guinea pigs with low (suboptimal) numbers of active (i.e., disease-inducing) cells prior to sensitization with either BP or whole CNS tissue in CFA. After BP/CFA sensitization, such animals show early (day 6) mild disease but subsequently remain completely healthy. We have examined these animals for the presence of suppressor T cells by transferring their cells to sensitized recipients. Despite use of many different cell populations (i.e. LNC, spleen cells, and PEC) at a variety of times, no suppressor cells could be detected in the suppressed animals. Clearly a mechanism other than suppressor cells is responsible for the suppression seen in these animals.

Inflammation: As described previously, antigen-specific T cells must be responsible for locating specific antigen and initiating lesion formation in the CNS, but that alone cannot be responsible for lesion development. Development of CNS lesions is due to the invasion of antigen non-specific cells. That cells other than BP-stimulated cells are involved in the transfer of EAE is obvious from our work with BP-sensitized guinea pig PEC. Previously, we had reported that culture of BP-PEC with specific antigen was sufficient for enhanced transfer

of disease. We now know that these cells require a second non-specific stimulant in culture. This stimulant is lipopolysaccharide (LPS). The requirement for LPS was not previously recognized since LPS is a common contaminant of most batches of the fetal calf serum (FCS) used in tissue culture. However, when FCS with very low levels of endogenous LPS is used in culture, BP-PEC cultured with BP are incapable of transferring disease. These cells do proliferate in response to BP (without added LPS). Only when exogenous LPS is added to culture can the cells transfer severe EAE. In fact, two aliquots of the cells can be exposed to the two stimulants separately. BP-PEC cultured only with BP transfer EAE poorly and BP-PEC cultured only with LPS are incapable of transferring disease. However, if both are injected into the same recipient, severe EAE results. It is clear that cells exposed to two separate stimulants in vitro can act synergistically in vivo to cause disease.

Chronic EAE: The most complicated type of EAE induced in guinea pigs is chronic EAE. Although it is not a good model for examining underlying immunologic mechanisms in EAE, it is an extremely important disease model for human MS. Lesions formed in chronic EAE are not the typical inflammatory lesions found in acute EAE but are demyelinating lesions similar to the lesions in MS. Since demyelination is responsible for the bulk of the permanent neurologic damage seen in MS patients, examination of the antigens responsible for and the mechanisms leading to demyelination is of critical importance. Our model of chronic EAE is induced by the technique described above, i.e., a suboptimal transfer of cells followed by sensitization with guinea pig spinal cord in CFA. Animals sensitized in this fashion develop various types of prolonged and sometimes relapsing-remitting disease. More importantly, many of the tissue sections examined reveal areas of extensive demyelination in the CNS of these animals. Our current experiments are aimed at defining the antigens in CNS tissue responsible for demyelination and the mechanisms by which such demyelination proceeds.

Significance to Biomedical Research and the Program of the Institute:

A clear understanding of the events responsible for generation of an immune response is necessary before intelligent intervention in the process is possible. This is particularly true of cell-mediated immune responses, including DTH. Delineating the role played by specific immunity, subsequent inflammation and, in the case of the CNS, demyelination, is critical to our understanding of various pathologic states. While in vitro testing is useful for detecting specific immunity, studies on specific immunity leading to inflammation must be done in vivo. EAE provides an invaluable system for delineating these responses. Furthermore, chronic EAE is the best available animal model of CNS demyelination and, coupled with studies in immunity and inflammation, should prove a useful model of immunopathologic diseases of the CNS, especially multiple sclerosis.

Our studies on strain specificity are of importance to multiple sclerosis (MS) research in humans. There is an association between certain major histocompatibility (MHC) loci and MS; one of these loci is the region of the MHC known to control the specificity of the immune response. Depending on their genetic makeup, different people may respond to different regions of the BP molecule which may vary in their encephalitogenicity; thus persons genetically programmed to respond to a particular region of the BP molecule might be more or less susceptible to disease.

Proposed Course:

Currently we are attempting to identify the soluble factors involved in the in vitro stimulation of BP-specific cells. While these antigen-specific cells are required for transfer of EAE, they do not appear to be sufficient for such transfer. In vivo there is a further requirement for cells stimulated in an antigen non-specific fashion to induce severe EAE. We are attempting to identify this second cell type and the mechanism by which these cells react synergistically with antigen-stimulated cells to transfer disease.

To date chronic demyelinating EAE has only been induced by sensitization with guinea pig spinal cord in CFA. We are currently inducing this disease by sensitization with BP in CFA in one site and other CNS antigens (in CFA) in a separate site. Preliminary data indicate that demyelination can be induced by this technique. Thus, the encephalitogenic sensitization (BP/CFA) can be physically separated from the sensitization responsible for demyelination. We can now examine the constituents of this second sensitization to determine the antigen(s) responsible for demyelination.

Publications:

Kies, M. W. and Driscoll, B. F.: Immunologic reactivity of myelin basic protein in inbred guinea pigs. In Peeters, H. (Ed.): Protides of the Biological Fluids. New York, Pergamon Press, 1982, pp. 115-118.

Iivanainen, M., Driscoll, B., Richert, J., Leon, M., Chu, A., Kies, M., Brown, B., Wallen, W., Madden, D., and Sever, J.: Oligoclonal IgG in the cerebrospinal fluid of guinea pigs with experimental allergic encephalomyelitis (41510). Proc. Soc. Exp. Med. 171: 272, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00903-06 LCM
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Improved Preparation of Human Myelin Basic Protein		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Gladys E. Deibler, Chemist, LCM, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Cerebral Metabolism		
SECTION Section on Myelin Chemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.5	PROFESSIONAL: 1	OTHER: 1.5
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The <u>isolation of human myelin basic protein (HBP)</u> has been modified to improve the quality of the preparation. HBP prepared by our standard large-scale procedure is always partially degraded and all preparations are contaminated with a <u>neutral proteinase</u> such that incubation at 37°C for 24 h causes complete <u>degradation of the BP</u>. BP extracted directly from human myelin has less enzyme activity but is nevertheless extensively degraded. Because of the enzymatic contaminant in the batch preparation, it is unsuitable for the further purification essential for preparation of purified BP-fragments. By modifying our current batch procedure, we have been able to prepare HBP from whole CNS with negligible amounts of enzyme activity. However, the purified HBP still contains more degradation products than other mammalian BPs prepared by the new procedure. The possible significance of these fragments <u>in vivo</u> is under investigation. </p>		

Project Description:Others Involved in this Project:

M. W. Kies Chief, Section on Myelin Chemistry NIMH, LCM

Objectives:

Modification of our large scale isolation procedure for myelin basic protein to eliminate significant proteolytic contamination in human and rat BPs.

Methods Employed:

In the past, basic protein has been prepared by a batch procedure from whole CNS of bovine, guinea pig, human, monkey, rabbit and rat. Proteolytic enzyme activity was essentially absent from bovine, guinea pig, monkey and rabbit preparations. Human and rat BPs were completely degraded after incubation for 24 h at 37°C. However, a newer more sensitive electrophoretic system in use now shows breakdown products which were not visible before. Bovine, guinea pig, monkey and rabbit BPs, when incubated for 24 h at 37°C show slight but significant breakdown.

In the earlier procedure, carboxymethyl cellulose was used to remove the BP by absorption from the bulk of the impurities. In the new procedure, the contaminants are removed by absorption on diethylaminoethyl cellulose at pH 9.0 leaving BP in solution. This absorption also removes the enzyme. Other steps in the isolation were unchanged.

Major Findings:

The new procedure is more rapid and yields a solution of HBP that can be applied directly to a carboxymethyl cellulose column for further purification by ion exchange chromatography. The final purified product, HBP (component 1) can be used for the preparation of peptides for biological studies. When component 1 was incubated at pH 8.0 at 37°C, for 24 h we found that even this highly purified preparation showed some degradation. The incubation of bovine BP (component 1), guinea pig BP (component 1), rabbit BP (component 1), and rat BP (component 1), all showed approximately the same amount of degradation when incubated under these conditions. However, each species of BP showed its own specific pattern of breakdown.

In order to determine if there were two enzymes contaminating HBP or if BP was susceptible to non-enzymatic hydrolysis, we determined the effect of pH and temperature on both activities. We found that the contaminating enzyme had a definite pH optimum at 7.00 and the optimum temperature for degradation of batch prepared BP was 47°C. As the temperature was increased beyond 47°C, the activity of the contaminating enzyme decreased. When the solution of HBP was placed in a boiling water bath for 30 min at pH 7.0 all activity stopped.

In contrast, the purified component 1 showed no pH optimum (i.e., more degradation occurred at pH 9 than at pH 7) and there was no temperature optimum.

In fact, boiling did not inhibit degradation at pH 9.0 - on the contrary, hydrolysis at boiling temperature increased with time. Therefore we concluded that the degradation of HBP (component 1) was not enzymatic but was due to the hydrolysis of susceptible bonds under the conditions used for incubation - 24 h at 37°C, pH 8.0, or for shorter periods of time at 100°C, pH 9.0.

Significance to Biomedical Research and the Program of the Institute:

The ability to isolate highly purified HBP (component 1) allows us to prepare peptides of known sequence and study the biological activity of different regions of the molecule.

Since HBP extracted from human myelin, also contains degradation products, and since the enzyme is not active in the conditions used for extraction, we assume that these products are present in situ or are formed during the myelin isolation. The origin and nature of these fragments may provide some insight into the abnormal metabolism which gives rise to BP peptides in CSF of patients suffering from demyelinating disease.

Proposed Course:

Because we have prepared HBP essentially free of enzymatic activity, we can now investigate the degradation products, which are still present. We are in the process of developing an HPLC method for purifying these fragments. Once we have them pure we will be able to identify their sequence by N-terminal, C-terminal and amino acid analyses. Similar studies will be carried out on fragments prepared by incubation of the old batch preparation. A comparison of the two sets of fragments should enable us to determine whether the fragments which accompany HBP to the final purification step were associated with BP in situ or were created during early steps in the isolation procedure.

Publications:

Deibler, G.E., Nomura, K., and Kies, M.W.: Limited digestion of guinea pig myelin basic protein and its carboxy-terminal fragment (residues 89-169) with Staphylococcus aureus V8 protease. J. Neurochem. 39: 1090-1100, 1982.

Deibler, G.E., Boyd, L.F., Bacon, M.L., Driscoll, B.F., and Kies, M.W.: Purification by HPLC of a Large Peptic Fragment of Myelin Basic Protein. Neurochem. Pathol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00931-10 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characteristics and Regulation of <u>S-Adenosylhomocysteine Hydrolase</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) G. L. Cantoni Chief, Lab. Gen. Comp. Biochem. LGCB NIMH		
COOPERATING UNITS (if any) Istituto Superiore di Sanita, Rome, Italy Istituto di Chimica Biologica, University of Rome, Rome, Italy		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Proteins		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3	PROFESSIONAL: 2.5	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>A large number of analogs of <u>adenosine</u> or of <u>adenosylhomocysteine</u> have been examined for their ability to function as inhibitors and/or substrates of <u>adenosylhomocysteinase</u>. The synthesis of analogs of adenosylhomocysteine by this enzyme <u>in vivo</u> may be used to form very potent and specific inhibitors of <u>trans-methylation</u> reactions. These analogs have been shown to have a wide range of important biological activities, including antiviral activity against a number of <u>RNA</u> and <u>DNA</u> viruses, <u>inhibition</u> of <u>viral transformation</u>, <u>inhibition</u> of <u>chemotaxis</u> in mouse macrophage cell lines, and <u>stimulation</u> of <u>cell differentiation</u> in a number of cell lines.</p> <p>The ability of several adenosine analogs to inhibit and/or act as substrates for the adenosylhomocysteine hydrolase has been examined both <u>in vitro</u>, using <u>purified enzyme</u> from <u>beef liver</u> and <u>hamster liver</u>, and <u>in vivo</u> for a variety of cell lines. The two most interesting compounds studied are <u>3-deazaadenosine</u> and <u>3-deazaaristeromycin</u>. Both are inhibitors of adenosylhomocysteinase, but only 3-deazaadenosine is a substrate for the enzyme. 3-Deazaadenosine, but not 3-deazaaristeromycin <u>inhibits chemotaxis</u> by a mouse macrophage cell line. <u>In vivo</u>, several methylation reactions, including <u>phospholipid methylation</u>, <u>protein lysine</u> and <u>arginine methylation</u>, and <u>protein carboxyl methylation</u> are inhibited to the same extent by both compounds. However, 3-deazaadenosine specifically inhibits synthesis of a small number of proteins, which may be due to inhibition of some reaction involved in <u>mRNA synthesis or processing</u>, such as <u>methylation</u> of the <u>5' cap</u>. Differences in specificities of these compounds can be used to search for the function of specific methylation reactions in chemotaxis, RNA synthesis, and other cellular processes.</p>		

Other Principal Investigators:

P. S. Backlund, Jr.	Staff Fellow	LGCB NIMH
R. R. Aksamit	Senior Staff Fellow	LGCB NIMH
A. Bozzi	Visiting Associate	LGCB NIMH
G. de la Haba	Research Scientist	LGCB NIMH

Other Investigators:

T. M. Caryk	Chemist	LGCB NIMH
S. Scarpa	Biologist, Istituto Superiore di Sanita, Rome, Italy	
R. Strom	Biochemist, Istituto di Chimica Biologica, Univ. of Rome, Rome, Italy	

Project Description:

As is well known, S-adenosylmethionine (AdoMet) is a key intermediate in biological transmethylation and transalkylation reactions. There are hundreds of reactions, each catalyzed by a specific enzyme, that utilize AdoMet as a substrate. It is obvious that the utilization of AdoMet in biological systems must be under regulatory controls, but at the present time little is known about the nature of these controls. It has been established that S-adenosylhomocysteine (AdoHcy), one of the products of transmethylation reactions that utilize AdoMet as methyl donor, is a competitive inhibitor of most reactions in which AdoMet participates. From the result of work in this and other laboratories, it has been proposed that the intracellular ratio of AdoMet/AdoHcy must be of key importance in the regulation of biological alkylation reactions, and that this ratio plays a role in determining the hierarchy of biological methylation reactions. In eukaryotes, AdoHcy is metabolized through a single metabolic pathway by S-adenosylhomocysteine hydrolase (AdoHcyase), an enzyme which catalyzes the reversible hydrolysis of AdoHcy to adenosine and homocysteine. Because of the central role of AdoHcyase in the metabolism of AdoHcy and in maintaining the ratio of AdoMet/AdoHcy, this enzyme has been under intensive study in this and other laboratories. Studies on purified AdoHcyase from several sources have shown that this is a complex enzyme, made up of four subunits, which bind NADH, adenosine and cAMP.

While the biochemical mechanisms of transmethylation reactions have been elucidated many years ago, largely as a result of the studies by Cantoni and his collaborators at NIH, the correlation between many methylation reactions and cellular functions remains obscure. For instance, the significance of the methylation of a variety of informational macromolecules, such as proteins and nucleic acids (DNA, ribosomal-, messenger-, viral and tRNA, etc.), or of complex polysaccharides, or even simpler compounds such as guanido acetic acid, nicotinamide, etc., is not immediately obvious and is the subject of much debate. It can be surmised that modulation of AdoMet/AdoHcy ratio would result in important physiological effects, which if correlated with biochemical data would help reveal the significance of specific methylation reactions.

In prokaryotes, the isolation of mutants has helped to analyze the functions of specific biochemical reactions. In eukaryotes, isolation of mutants is more difficult, so other approaches have been devised, such as using inhibitors to block specific reactions. Since AdoHcyase is the only enzyme to metabolize AdoHcy in eukaryotes, inhibition of this enzyme by analogs can be used to alter the ratio of AdoMet/AdoHcy in the cell. We decided some years ago to take advantage of this fact and initiated a long range experimental project designed to study in depth the properties of AdoHcyase, and then to develop a series of specific inhibitors of this enzyme. As a result of these studies on the properties of AdoHcyase, we have established that the use of specific inhibitors makes it possible to alter the intracellular levels of AdoHcy and/or to accumulate intracellularly congeners of AdoHcy of the general formula S-purinyllhomocysteine (PurHcy). By using these inhibitors, it is possible to modulate the AdoMet/AdoHcy and/or AdoMet/PurHcy ratio in different cellular systems, and to examine the consequences of these changes on cellular functions.

Our studies, confirmed and extended in other laboratories, have shown that inhibitors of AdoHcyase may be divided into two groups: a) irreversible or suicidal inhibitors, and b) competitive inhibitors that inhibit the enzyme reversibly. This second group can be further classified into two subgroups; those inhibitors which can be utilized as substrates by the enzyme and those inhibitors which are not substrates. Several compounds have been examined which are irreversible inhibitors of AdoHcyase, and include the compounds 9- β -D-arabinofuranosyladenine (Ara-A), 3-deaza-9- β -D-arabinofuranosyladenine (3-deaza-Ara-A), and 2-chloroadenosine. Ara-A has been used by others in chemotherapy for cancer patients. 3-Deaza-Ara-A and 2-chloroadenosine might be expected to have clinical effects similar to Ara-A, since they produce similar inhibition of AdoHcyase. Of the many reversible inhibitors tested, two compounds have been extensively studied in this laboratory as prototype compounds of this group; 3-deazaadenosine (3-deaza-Ado) and 3-deaza-aristeromycin (3-deaza-Ari). 3-Deaza-Ado is a potent competitive inhibitor of AdoHcyase with K_i of 5-8 μ M, and as a substrate it is about equivalent to the natural substrate, adenosine with a similar affinity for the enzyme. In contrast to 3-deaza-Ado, 3-deaza-Ari is not a substrate for AdoHcyase, but it is a very potent competitive inhibitor, with K_i of 2.0 nM for the hamster liver enzyme. Neither compound is a substrate for either adenosine kinase or adenosine deaminase.

In vivo, administration of 3-deaza-Ado to laboratory animals or cells in culture results in the accumulation of both 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) and AdoHcy. The accumulation of 3-deaza-AdoHcy can be increased by addition of homocysteine, due to AdoHcyase acting in reverse of the normal hydrolytic direction. Administration of 3-deaza-Ari brings about an increase in AdoHcy, due to the inhibition of AdoHcyase. Since 3-deaza-Ari is a potent inhibitor of AdoHcyase, the amount of AdoHcy which accumulates, reflects the rate of transmethylation reactions, and not the catalytic rate of AdoHcyase. Differences between species were observed for the metabolite levels formed after treatment with these compounds. Administration of 3-deazaadenosine to rats produced accumulation of both 3-deaza-AdoHcy and AdoHcy, however, the same treatment in hamsters resulted only in an accumulation of 3-deaza-AdoHcy. Examination of the kinetic properties of the enzyme from rat and hamster liver revealed that the K_m for AdoHcy is ten times smaller for the hamster enzyme than for the rat. This could explain the lack of AdoHcy accumulation in hamster liver.

It would be expected that the intracellular accumulation of AdoHcy and/or 3-deaza-AdoHcy, with the accompanying changes in the AdoMet/AdoHcy ratio, would result in the inhibition of a number of transmethyases. This should cause an increase in the intracellular level of AdoMet (as a consequence of its under-utilization) and in a decrease in the intracellular concentration of many methylated intermediates. We have been able to verify this prediction, demonstrating a striking decrease in the amount of many methylated compounds, including methylated phospholipids, methylated proteins, and creatine in the liver.

Studies in this and other laboratories on a large number of analogs of AdoHcy analogs have demonstrated a wide range in the sensitivity of different trans-methyases to inhibition by these compounds in vitro. Unfortunately, cellular membranes are relatively impermeable to AdoHcy and its analogs, so it has been difficult to take advantage of the specificities of these analogs in vivo. However, the capacity of AdoHcyase to synthesize AdoHcy analogs in vivo, as has been shown with 3-deaza-Ado, demonstrates the exciting possibility of synthesizing potent and specific methylation inhibitors intracellularly.

Since 3-deazaaristeromycin has such a low K_i for AdoHcyase, high concentrations of this compound should effectively block the enzyme, preventing the conversion of AdoHcy to adenosine and homocysteine. Comparison of the effects of 3-deaza-Ado and 3-deaza-Ari on the replication of RAW264 cells showed that, at sufficiently high concentrations, 3-deaza-Ado was cytolytic after one day and that 3-deaza-Ari was cytostatic. Micromolar homocysteine reversed the cytostasis of 3-deaza-Ari, but did not reverse the cytotoxicity of 3-deaza-Ado. The cytotoxicity of 3-deaza-Ado is likely mediated by 3-deaza-AdoHcy, while on the other hand, cytostasis of 3-deaza-Ari was due to a profound inhibition of AdoHcyase. Since AdoHcy is the only cellular source of homocysteine, cells incubated with 3-deaza-Ari cannot recycle methyltetrahydrofolate and regenerate tetrahydrofolate for use in de novo synthesis of purines and pyrimides. This condition is similar to the situation with vitamin B₁₂ deficiency, which inactivates methionine synthase, and causes methyltetrahydrofolate to accumulate. In addition, it would be expected that cells incubated with 3-deaza-Ari would contain less cystathionine, an amino acid without a known function that is found in high concentration in the brain. These findings could have clinical significance in situations where AdoHcyase is inhibited such as the administration of Ara-A and patients with adenosine deaminase deficiency.

Comparison of the biological effects of 3-deaza-Ado and 3-deaza-Ari has made it possible to attribute some of the differences in specificity to the finding that 3-deaza-AdoHcy is a more potent and specific inhibitor of some transmethylation reactions than AdoHcy. We have found that macrophage chemotaxis is specifically inhibited by the intracellular formation of 3-deaza-AdoHcy, brought about by treatment of the cells with 3-deaza-Ado, while chemotaxis is unaffected by accumulation of AdoHcy by treatment with 3-deaza-Ari. Both 3-deaza-Ado and 3-deaza-Ari bring about a significant inhibition of methylation of phosphatidylethanolamine in these cells, which rules out a requirement for this reaction in chemotaxis. In the same manner, both compounds inhibit protein lysine and arginine methylation and carboxylmethylation to the same extent. We have further shown that inhibition of chemotaxis by 3-deaza-Ado is correlated with inhibition of the synthesis of specific proteins which are not inhibited by 3-deaza-Ari.

Both 3-deaza-Ado and 3-deaza-Ari inhibit various RNA and DNA viruses, however, the sensitivity of various viruses to these two drugs is different. In addition, the synthesis of cellular mRNA in macrophage cells is inhibited to a greater extent with 3-deaza-Ado than with 3-deaza-Ari. The specific reaction(s) involved in inhibition of RNA synthesis has not been identified.

Both 3-deaza-Ado and 3-deaza-Ari can stimulate cell differentiation in a number of cell lines, suggesting that a methylation reaction may be involved in altering gene expression in differentiation. Again, there are differences between these two compounds in their ability to induce differentiation in different systems. In collaboration with Drs. Scarpa, Strom, and Bozzi, it was found that 3-Deaza-Ado will increase the rate of myoblast fusion to form myotubes when the cells are placed in a permissive fusing medium. In addition, non-fusing variants of myoblast cells, which normally do not fuse in a permissive fusing medium, will fuse when 3-deaza-Ado is added. Work from several other laboratories has suggested that DNA methylation may be involved in expression of specific genes. It is possible that 3-deaza-Ado may cause differentiation of these cells by inhibiting DNA methylation. However, since 3-deaza-Ado also inhibits a number of other methylation reactions, further work will be required to identify the mechanism for this effect of 3-deaza-Ado.

In a series of recent studies in Europe and in this country, it has been found that AdoMet, given parenterally to depressed patients produced rapid and remarkable improvement in the clinical picture. These studies indicate that AdoMet has approximately the same antidepressant activity as the standard tricyclics, such as imipramine, aminotripyline, etc. It is noteworthy, however, that administration of AdoMet is not accompanied by any toxic side effects, and thus, this mode of therapy may represent a considerable improvement over the therapeutic regimens currently in use.

The mechanism of action of AdoMet in depressive illness is unknown. It should be pointed out, however, that the dose of AdoMet found to be effective in the management of clinical depression (200-400 mg/i.v./day) is very small compared to the daily flow of methionine through AdoMet. Human adults synthesize and metabolize about 20 millimoles of AdoMet/day, or 20-40 times the dose used in clinical trials.

Significance to Biomedical Research and the Program of the Institute:

Studies of the AdoHcyase and its inhibitors are important to understanding the regulation and function of biochemical transmethylation, and have possible clinical applications in the development of specific inhibitors for certain methylation reactions. Since AdoMet dependent methylation reactions are involved in the synthesis of so many compounds, including DNA, RNA, proteins, lipids, and neurotransmitters, the regulation of these reactions can alter many cell functions. Inhibitors of methylation reactions have been shown to affect cell differentiation, leukocyte chemotaxis, and virus replication. However, the specific reaction(s) involved are not clear, since most methylation inhibitors are not specific for just one reaction. The possible clinical applications could be in the development of compounds for use in chemotherapy, immunosuppression, and antiviral drugs. Because of the important role of methylation in neurotransmitter synthesis, these compounds could have important effects on brain function as well.

Proposed Course of Research:

Studies on several inhibitors will continue in order to determine specific mechanisms of inhibition, and to determine correlations between inhibition of specific reactions and the physiological effects of these compounds. Much of the work will focus on methylation reactions involved in leukocyte chemotaxis, and in RNA and protein synthesis. The differences in inhibition of lipid and protein methylation by the various compounds will continue to be examined.

Publications:

Kim, I.-K., Aksamit, R.R., and Cantoni, G.L.: Mechanism of the cytostatic activity of 3-deazaaristeromycin, an inhibitor of adenosylhomocysteine hydrolase. J. Biol. Chem. 257: 14726-14729, 1982.

Cantoni, G.L., Aksamit, R.R., and Kim, I.-K.: Methionine biosynthesis and vidarabine therapy. N. Engl. J. Med. 307: 1079, 1982.

Kim, I.-K., Zhang, C.-Y., Chiang, P.K., and Cantoni, G.L.: S-Adenosylhomocysteine hydrolase from hamster liver: purification and kinetic properties. Arch. Biochem. Biophys., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00934-11 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Biochemical Basis of Narcotic Drug Action		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) W. A. Klee Research Chemist LGCB NIMH		
COOPERATING UNITS (if any) Laboratory of Vision Research, NEI; Laboratory of Neurophysiology, NINCDS; Laboratory of Chemistry, NIADDK; Laboratory of Developmental and Molecular Immunology, NICHD; and National Institute of General Medical Sciences		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Proteins		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <u>Opiate receptor mediated inhibition of adenylate cyclase requires the presence of a GTP binding protein, N_i in order to be expressed. Pertussis toxin catalyzes the ADP-ribosylation of N_i and thereby attenuates the inhibitory actions of opiate, and other receptors on adenylate cyclase. Adenylate cyclase inhibition is accompanied, and probably caused by increased GTP hydrolysis which is also blocked by toxin treatment. A third role of N_i is in the control of opiate binding affinity and this too is lost on treatment with the toxin. Affinity labelling of opiate receptors with fentanylisothiocyanate (FIT) has led to the identification and partial purification of a 58,000 M_r glycoprotein subunit of the receptor. Active opiate receptors have been transferred from solution to liposomes as a first step in their reconstitution.</u>		

Other Investigators:

L. Hjelmeland	Senior Staff Fellow	LVR NEI
K. C. Rice	Research Chemist	LC NIADKDK
A. E. Jacobson	Research Chemist	LC NIADKDK
C. Zioudrou	Visiting Scientist	LGCB NIMH
J. Barker	Chief, Lab. of Neurophysiology	LNP NINCDS
F. W. Sweat	Research Scientist	LGCB NIMH
W. F. Simonds	Pharmacology Research Associate	NIGMS
B. Tocque	Guest Worker	LGCB NIMH
G. Milligan	Visiting Fellow	LGCB NIMH
R. Sekura	Research Chemist	LDMI NICHD
A. Pfeiffer	Guest Worker	LGCB NIMH

Project Description:

Over the past several years we have been engaged in the characterization of the interaction between opiate receptors and adenylate cyclase in a cultured neuronal cell line, NG108-15. The cell has a large number of opiate receptors which function to inhibit adenylate cyclase and thereby lower cAMP levels. In analogy to the addictive process, the cells become tolerant to, and dependent upon, opiates after prolonged exposure. This adaptive response is due to a gradual increase in adenylate cyclase activity which serves to maintain normal cAMP levels in the continued presence of opiates. In the past year, we have made significant progress in our understanding of the detailed mechanism of some of these opiate actions. Moreover, work in this and other laboratories has shown that the cyclic nucleotide linked mechanism of opiate action is operative in brain as well as in the cultured cell system.

As a first step towards the purification and reconstitution of the cellular constituents involved in opiate action, we solubilized opiate receptors from several sources using the zwitterionic detergent, CHAPS. Receptors which reversibly bind opiate ligands with the appropriate specificity were isolated from membranes of NG108-15 cells, brain tissue (both rat and beef), and human placenta. Each of these receptor preparations behaves as a macromolecular complex of Stokes radius near 7 nm and contains protein as an essential constituent.

Rice, Jacobson and their colleagues have recently prepared a series of opiate ligands containing alkylating functions so that they might serve as covalent affinity labels of the receptors. We have studied the biological activities of these substances and found several of them to be uniquely selective irreversible ligands for either μ or δ receptor subtypes. We have prepared several of these substances in radioactively labelled form so that they may serve as tracers for receptor characterization and purification. The most useful of these compounds, to date, is fentanylisothiocyanate (FIT) which covalently binds to the (exclusively) δ receptors of NG108-15 membranes. Interestingly, the material behaves as an agonist even when covalently bound. The radioactive material can be shown to bind specifically only to a 58,000 M_r glycoprotein. Because this binding is blocked by active opiates and not by their inactive enantiomers, we believe the protein to be the recognition subunit of the opiate receptor. It has been purified by a combination of wheat germ lectin chromatography and electrophoresis. Antibodies to FIT

have been prepared and purified by affinity chromatography. These have been coupled to Sepharose and are now being exploited as an affinity matrix to effect further purification of the FIT labelled receptor subunits. The antibodies have also been found to be useful as extremely sensitive stains for FIT labelled receptors in conjunction with second antibodies and peroxidase.

The guanine nucleotides GDP, GTP and guanosine-5'(β,γ -imido)triphosphate inhibit binding of opiates and opioid peptides to receptors solubilized from neuroblastoma x glioma NG108-15 hybrid cells. The inhibition requires sodium ions and reflects a decrease in affinity of receptors for opioid ligands. These observations are consistent with the suggestion that solubilized receptors are complexes composed of an opiate binding protein and a guanine nucleotide regulatory component. Indeed, when such preparations are subjected to gel exclusion chromatography, opiate binding activity migrates together with the guanine nucleotide regulatory proteins of adenylate cyclase. Opiate inhibition of adenylate cyclase activity was shown to result from a more direct stimulation of GTP hydrolysis catalyzed by an inhibitory receptor coupled GTPase in neuronal membranes. Neither opiate stimulation of the GTPase nor inhibition of adenylate cyclase is observed in detergent solutions of neuronal membranes. Thus, even though receptor binding in such solutions is GTP sensitive, some component of the receptor-GTPase coupling mechanism has become limiting. A major goal of our work is the reconstitution of coupling of receptor occupancy to adenylate cyclase inhibition (and GTPase stimulation) in solubilized preparations. Functional reconstruction of the coupled activities is necessary to determine the numbers of essential constituents and the role which each component plays. A first step towards reconstitution is our recent demonstration that functional opiate receptors can be transferred, by polyethylene glycol induced fusion, from membranes of NG108-15 cells to those of other cells.

Solubilized receptor preparations have not yet been reconstituted in this way. We have, however, recently had some success in incorporating solubilized receptors into phospholipid vesicles and at the same time maintaining both binding activity and GTP sensitivity of binding. Fusion of such active liposome preparations with the appropriate adenylate cyclase-membrane preparation should, we believe, lead to reconstitution of coupling. Experiments designed to test this hypothesis are currently in progress.

It has recently become clear that there are two GTP binding regulatory proteins associated with coupling receptors to adenylate cyclase. These are N_s which modulates stimulatory (β -adrenergic or dopamine, for example) receptor activity and N_i which modulates the activity of inhibitory receptors. These regulatory proteins are modified both in structure and function by the bacterial toxins causing cholera (N_s) and pertussis (N_i). We have recently found that both toxins affect N_i activity to some extent and also that toxin catalyzed ADP-ribosylation of one regulatory protein changes the susceptibility of the other to modification by its toxin. These results have led us to suggest a mechanism for receptor mediated regulation of adenylate cyclase in which the two GTP binding proteins interact with each other as well as with adenylate cyclase. We have also developed assays for N_i activity based upon reconstitution of opiate receptor affinity and activity with pertussis toxin treated membranes, and are in the process of purifying this protein with the aid of such assays.

Several years ago, we showed that cells accommodate to chronic exposure to opiates by developing an increased adenylate cyclase activity. This homeostatic mechanism is elicited by all opiate agonists. In addition, we have recently found that highly efficacious agonists, such as etorphine or enkephalin, also cause a down regulation of receptor numbers whereas morphine does not do so. Down regulation of receptors is thus not necessary for homeostasis but occurs with some opiates only. Changes in N_1 function are not responsible for down regulation because other inhibitory receptors, such as α_2 -adrenergic, are unaffected by etorphine treatment. We have recently refined our assay procedures so as to be able to measure adenylate cyclase and GTPase activities simultaneously with membranes prepared by a rapid isolation procedure. We are now, therefore, able to determine whether the increased adenylate cyclase activity resulting from chronic opiate treatment reflects a stable modification of adenylate cyclase, GTPase activity or yet another activity.

Significance to Biomedical Research and the Program of the Institute:

A major problem in biology is understanding the mechanism of signal-response coupling across cell membranes. Cells communicate with one another and with their environment largely through chemical messengers which are sensed by cell surface receptors and thereby elicit other chemical changes within the cell. The opiates, and related substances, are important transmitters of information in the nervous system. An understanding of how brain cells transmit and use such information is essential to the design of rational therapy for mental illness.

Proposed Course of Research:

We hope to continue to characterize receptors and their associated enzymatic apparatus in both structural and functional terms. We plan to purify each of the proteins of the system to homogeneity and hope to ultimately find ways of recombining them in such a way as to reconstitute each of the functions of the receptors. Because of the low abundance in which receptors are found, full purification will almost certainly require the use of monoclonal antibodies, and we are currently attempting to prepare such materials. Full structural characterization will require cloning of the receptor gene(s) and we hope to use both antibodies and partial sequence information to carry out such cloning experiments.

Publications:

Koski, G., Streaty, R.A., and Klee, W.A.: Modulation of sodium-sensitive GTPase by partial opiate agonists. J. Biol. Chem. 257: 14035-14040, 1982.

Klee, W.A., Simonds, W.F., Sweat, F.W., Burke, T.R., Jr., Jacobson, A.E., and Rice, K.C.: Identification of a M_r 58,000 glycoprotein subunit of the opiate receptor. FEBS Lett. 150: 125-128, 1982.

Zioudrou, C., Varoucha, D., Loukas, S., Streaty, R.A., and Klee, W.A.: Photolabile ligands for opiate receptors. Life Sci. 31: 1671-1674, 1982.

Hjelmeland, L.M., Klee, W.A., and Osborne, J.C., Jr.: A new class of nonionic detergents with a gluconamide polar group. Anal. Biochem. 130: 485-490, 1983.

Rice, K.C., Jacobson, A.E., Burke, T.R., Jr., Bajwa, B.S., Streaty, R.A., and Klee, W.A.: Irreversible ligands with high selectivity toward δ or μ opiate receptors. Science 220: 314-316, 1983.

Zioudrou, C., Varoucha, D., Loukas, S., Streaty, R.A., and Klee, W.A.: Photolabile opioid derivatives of D-Ala²-Leu⁵-enkephalin and their interactions with the opiate receptor after photolysis. J. Biol. Chem., in press.

Jacobson, A.E., Bajwa, B.S., Streaty, R.A., Klee, W.A., and Rice, K.C.: Probes for narcotic receptor mediated phenomena. 5. Narcotic antagonist irreversible ligands based on endoethenotetrahydrooripavine. Life Sci., in press.

Loukas, S., Varoucha, D., Zioudrou, C., Streaty, R.A., and Klee, W.A.: Opioid activities and structures of α -casein derived exorphins. Biochemistry, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00935-16 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effects of Viruses and Plasmids on the Biochemistry of Living Organisms		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) C. R. Merrill Senior Research Scientist LGCB NIMH		
COOPERATING UNITS (if any) Laboratory of Molecular Biology, NCI Laboratory of Clinical Science, NIAAA		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Proteins		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The primary goal of this study is to examine the role of <u>viral integration</u> and <u>plasmids</u> in Alzheimer's disease and the process of <u>aging</u> and <u>senescence</u>. Recent development of techniques to detect chromosomally integrated viral genomes had encouraged us to apply these methodologies to investigate human diseases. The possibility of an association between a viral genome integrated into the <u>chromosome</u> and the occurrence of diseases inherited in a dominant manner, such as <u>familial Alzheimer's disease</u>, is currently being examined.</p> <p>Integration of viral related <u>nucleotide sequences</u> in the genomic DNA of man is being studied using <u>fibroblast cell lines</u> established from individuals belonging to a family with histologically confirmed Alzheimer's disease. Tissues from this Alzheimer pedigree are also being utilized along with tissues obtained from young and aged normal individuals to examine amplification and excision events in the <u>mitochondrial genome</u> as a function of the aging. Since excision can result in the <u>synthesis</u> of circularized mitochondrial plasmids, (the major factor in senescence of some fungal strains) experiments are being conducted to screen for these free mitochondrial plasmids and an attempt is being made to determine their relationship with the state of <u>senescence</u>.</p>		

Other Investigators:

M. Gottesman	Chief, Biochemical Genetics Section	LMB NCI
S. L. Adhya	Geneticist	LMB NCI
D. Goldman	Chief, Unit on Molecular Genetics	LCS NIAAA

Project Description:

The methodologies for studying viral genes and their expression in host cells have developed rapidly over the past decade. The extension of these methodologies to study dominant genetic diseases, such as familial Alzheimer's disease, for viral genetic information is utilizing restriction endonucleases, electrophoresis, and DNA-DNA hybridization. At the present time, skin biopsies from almost 70 individuals belonging to a family with histologically confirmed Alzheimer's disease have been taken. These biopsies have been used to establish fibroblast cell lines which now have been grown up in sufficient quantity to allow extraction of human DNA for each line.

These DNAs are being screened with biotin-labelled viral probes, which are synthesized from the nucleic acid of selected viruses. The viruses used include: human - herpes virus, adenovirus, poliovirus, measles virus, cytomegalovirus, Epstein-Barr virus and BK virus; primate - SV40, baboon type-C virus, and simian sarcoma virus; and murine - Rauscher murine leukemia virus, Moloney murine sarcoma virus and mouse mammary tumor virus. These viruses were selected for their potential to detect related sequences in the human genome. The choice of human viruses is obvious as is the selection of a few primate viruses on the basis of the evolutionary relatedness of the two species. Murine viruses are also included because of the finding of 11-28% nucleic acid homology between some primate and murine viruses.

The biotin-labelling technique developed by David Ward and others is utilized rather than the more conventional radioactive labelling with ^{32}P . This method has been improved to the point where it is now capable of detecting single copy sequences in the eucaryotic genome as radiolabelled probes. The technique also has several advantages over the use of ^{32}P to radioactively label. The biotin-labelled probes are chemically stable (^{32}P has a half-life of only 14 days) and can be used over long periods of time (1-2 years) with reproducible results. The hybridization times for the detection of unique gene sequences can be reduced significantly. Additionally, the colorimetric visualization which is used with this procedure provides superior resolution over autoradiography with a ^{32}P -labelled probe.

Hybridization of a viral probe with a DNA restriction fragment may indicate the presence of virally related sequences in the cell lines. Since the fibroblasts were originally obtained from individuals comprising an extensive pedigree of familial Alzheimer's disease, the presence of chromosomally integrated viral genetic information could then be followed for genetic transmission throughout the family.

Many mammalian species contain endogenous viral information which usually remains "silent" due to either repression of the virus by the cell or integration of only a portion of the viral genome. The presence of these endogenous viral sequences in a wide range of animal species indicates their evolutionary preservation, and

according to some, such genetic information might provide functions with a selective advantage to the species possessing them. These endogenous viral genes also have the potential to be turned on by environmental agents or derepressed in an aging cell resulting in cell transformation and for these reasons it is imperative to examine the human genome for sequences related to these viruses.

Previous studies have looked for homology between DNA extracted from a cell and the DNA of a viral probe by reassociation kinetics. Using these methods, free viral nucleic acid could not be distinguished from integrated viral sequences. However, new techniques involving restriction endonuclease digestion, Southern blotting (Southern, 1975) followed by hybridization of a viral probe, allow this project to examine whether the cellular DNA of man contains integrated virus related sequences. Additionally, the sites of integration can also be examined. Integration sites may prove to be useful as polymorphic markers in the mapping of the genetic loci on the human chromosomes. This information may also aid in the understanding of gene regulation.

A number of disease-associated tissues have been examined for the presence of human viral sequences. Poliovirus type I and type II were used to study amyotrophic lateral sclerosis (Miller *et al.*, 1980) with negative results. Human cytomegalovirus (CMV) was used to probe DNA from the brains of patients with schizophrenia (Aulakh *et al.*, 1981) and multiple sclerosis (Aulakh *et al.*, 1980). Both of these hybridization analyses failed to detect any virus related genetic information complementary to the CMV probe. Herpes simplex virus (HSV) has also been used extensively to search for viral nucleotide sequences in brain tissue from patients with multiple sclerosis (Aulakh *et al.*, 1980), idiopathic Parkinson's disease (Wetmur *et al.*, 1979), and senile and presenile dementias of Alzheimer and Pick (Middleton *et al.*, 1980), again with negative findings. Neoplastic tissue has also been analyzed with several viral probes. One hundred and sixty-six tumors representing about 50% of all cancer types in the United States were extracted by Wold and his colleagues (1978) and hybridized with BK virus, a human papovavirus. They were unable to detect BKV sequences in the DNA from any of the human tumors. However, these studies were all done using liquid DNA-DNA hybridization which is not as sensitive as the techniques being utilized in this project. One study which employed Southern blotting (a technique we are using) of human tonsil DNA digested with Eco RI followed by filter hybridization with group human adenoviruses (Green *et al.*, 1979) found that all tonsils showed restriction fragments which might indicate adenovirus sequences integrated into tonsil DNA. Other investigations which indicate positive results include the identification of integrated hepatitis B virus DNA in a human hepatocellular carcinoma cell line Chakraborty *et al.*, 1981).

Based on this wealth of conflicting data, it seems imperative to approach this concept with the latest techniques. Alzheimer's disease is a good candidate for such research since evidence has implicated incomplete, latent or unconventional virus infections as a possible primary pathogenetic event. Possibly the disease state is a result of age-dependent relaxation of gene repression which could lead to the expression of specific viral genes not normally expressed. This relaxation of gene control has been previously suggested in diseases which may be associated with slow viruses.

Given that endogenous viruses have been found in a wide variety of vertebrate spe-

cies, such as reptiles, birds, rats, mice, pigs, cats, and primates, it seems likely that this project will uncover the presence of some virally related nucleotide sequences in the genomic DNA of man. Detection of integrated viral genes in human DNA will be of major importance in many fields of research and may spark increased analysis of virus associations in disease states.

Another approach to the study of diseases associated with aging, such as Alzheimer's, has been suggested by the work done with senescence in fungi. It appears that in fungi, senescence is caused by mutations affecting extrachromosomal genetic factors. This conclusion was drawn from three lines of evidence. The first evidence derives from the transmission of senescence in crosses of Podospora (Ricet, 1957; and Marcou, 1961). These researchers found that senescence is transmitted by the female parent since senescent male clones crossed with normal clones produced normal progeny, whereas, when the female parent was senescent, both normal and senescent progeny resulted. This pattern indicates that the genetic factor for senescence in fungi is not present in the nuclear chromosomal material but must be located in the cytoplasm of the cell.

The second line of evidence for the extrachromosomal location of senescence is derived from their "infectious nature." Normal clones of Neurospora become senescent following a transient fusion with senescent clones without acquiring any nuclear genes from the senescent strain (Bertrand and Pittenger, 1969). The state of senescence can also be transferred in Podospora without the relocation of nuclei or nuclear material.

The final and most convincing evidence was provided by microinjection experiments with Neurospora (Garnjobst et al., 1965; and Diacumakos et al., 1965). When normal clones were injected with purified nuclei or DNA from senescent cultures, no signs of senescence appeared. However, when the injected material was composed of cytoplasm or purified mitochondria from senescent cells, many of the resultant clones became senescent following several days of growth. These studies illustrated not only the extrachromosomal nature of the genetic factor but indicated that senescence is associated with the mitochondria. A model has been proposed for fungi, in which, the senescent state is triggered by mutational events which affect the mitochondrial genome. These defective mitochondria then multiply during the progression of senescence and eventually outnumber the normal mitochondria which leads to vegetative death.

Recent research into senescence in fungi has substantiated the proposed model and illuminated the specific mutational event which appears to initiate symptoms of senescence. Vierny and her colleagues (1982) have discovered a 2.6kb region in the mitochondrial genome of Podospora anserina which undergoes amplification. This sequence may also be excised out of the genome and exist as a free circularized sequence or plasmid. At the final stage of senescence, these plasmids constitute virtually all of the DNA found in senescent mitochondria. Mutant strains whose mitochondrial genomes lack the 2.6kb region display an increased longevity and may actually escape senescence. Free plasmids have also been found to migrate to the nucleus and become integrated into the nuclear genome (Wright and Cummings, 1983). This process might then promote instability and degeneration of the nuclear chromosomes.

The relationship between aging and changes in the mitochondrial genome is begin-

ning to be investigated in more complex species. It has been shown that there is an increase in circular dimers in tissues from senescent mice (Piko and Matsumoto, 1977). Mitochondrial DNA isolated from liver mitochondria from young and aged rats by cesium chloride, ethidium bromide isopycnic density gradient centrifugation revealed a novel band in 75% of the older animals (Murray and Balcarage, 1982).

Human cell lines and tissues (especially the brain) are being examined for mitochondrial mutational events associated with aging and senescence. The tissue mitochondrial DNA is purified, digested by restriction enzymes and analyzed for amplification or excision events. The cytoplasmic fraction is screened for free plasmids which may have originated in the mitochondria. DNA extracted from whole tissues or cells are being probed with sequences cloned from young mitochondria to determine if nuclear integration of plasmids is a factor in the senescence of humans.

Currently, we are in the process of cloning mitochondrial DNA sequences derived from human platelets. These cloned sequences will be useful to probe the DNA derived from human tissues representing various age groups. Additionally, they can be used to examine the large family pedigrees that have been established in cell culture. Should the process of senescence in mammalian species resemble the model proposed for fungi, we have also obtained the senescent plasmids of Podospora anserina from David Cummings, of Colorado, for use as potential probes.

Significance to Biomedical Research and the Program of the Institute:

Molecular genetic studies will prove to be a key to the understanding of normal brain function and the pathogenesis of neuropsychiatric diseases. There are a number of genetic neuropsychiatric diseases for which trait-specific molecular markers are lacking and for which the pathogenesis is largely unknown. Prominent among these is familial dominant Alzheimer's disease, the major focus of this project.

Because the prevalence of severe dementia in the Northern European population of age 65 or older is 4-5% and the prevalence of milder dementia is 11-12%, more than 1 million Americans are probably afflicted with severe dementia and 3 million have mild or moderate dementia. Cognitive dysfunction in old age does not occur inevitably; it is a consequence of pathological processes. Of demented patients, 50% show the neuropathological changes of Alzheimer's disease: neurofibrillary tangles, senile plaques and granulovacuolar bodies.

The diagnosis of Alzheimer's disease is presently clinical. Other causes of chronic cognitive deterioration such as toxins, nutritional deficiencies, infections, endocrine disturbances, cerebral tumors, arterial disease and normal-pressure hydrocephalus must be ruled out. In elderly patients, depression frequently masquerades as dementia. Definitive diagnosis currently depends on the histological study of brain tissue. Brain neurochemical findings have stimulated investigators to attempt treatment by manipulating cholinergic neurotransmission and approaches might be made to the treatment of other dementias. There is a definite need for a better method for diagnosing Alzheimer's disease.

The cause of Alzheimer's disease is unknown. It may be a slow infectious agent such as scrapie in sheep or Kuru and Creutzfeldt-Jakob disease in man. Psychiatric and neurologic sequelae may follow years after viral infection (as with measles and subacute sclerosing panencephalitis and with influenza and postencephalitic Parkinson's disease). The paired helical filaments of neurofibrillary tangles and neuritic plaques in Alzheimer's disease could be due to the interaction of viral protein with cell cytoskeletal protein which occurs during viral replication. A factor from brain and Alzheimer patients assembled neurofilaments into paired helical filaments and was cytopathic in neuronal cell cultures. The factor was RNase and protease sensitive but DNase resistant. Brain suspension from patients with familial Alzheimer's disease showed cell-fusing activity in 59% of cases, a level similar to that for Creutzfeldt-Jakob disease; only 3 of 17 sporadic Alzheimer cases (17%) showed fusion activity. Like the Moloney leukemia virus, in the experiments of Rudolph Janish, the putative virus or other infectious agents might integrate into the human germ cell genome, be transmitted in autosomal dominant fashion and show tissue specific expression of effects. We are searching for viral sequences as genetic markers and as potential disease causative agents.

The strongest evidence for a genetic factor in Alzheimer's disease is the fact that all individuals with Trisomy 21 develop the brain pathology of Alzheimer's disease if they live to their late 20's or 30's. Based on this fact, our laboratory has approached the molecular basis of Alzheimer's disease by attempting to identify (using high resolution protein two-dimensional electrophoresis) proteins coded for or modulated by Chromosome 21.

Integrated viral sequences are not only of interest in themselves but may often behave as polymorphisms. Molecular polymorphisms are genetic variants with an allelic frequency of greater than 1% in the normal population, and serve as diagnostic markers and molecular probes. When a polymorphic locus is close to a disease locus, the two loci will rarely be separated by recombination and linkage may be established. Mathematical analysis indicates that with the current clinical polymorphic markers available (about 30) approximately 20% of the human genome is currently covered at a genetic linkage distance of 10 centimorgans. Discovery of a sufficient number of polymorphic marker loci will allow the construction of a high resolution map of the human genome. Such a map will allow most genetic diseases which are primarily monogenic to be localized to a genomic subregion and will provide additional molecular probes for investigations of pathogenesis.

Studies on the role of mitochondria in diseases associated with aging may provide needed insight into the process of senescence. Every organism undergoes the progression of aging, but little is known about the events which trigger the decline. Recent investigations have indicated that mutations in the mitochondrial DNA, especially in the brain, may be associated with aging. Such changes in the critical energy supplying organelle of a cell are quite capable of resulting in profound effects.

The model proposed for the fungi may be simplistic when compared to the complexity of mammalian systems. However, considerable evidence has been accumulated on involvement of the mitochondrial genome in aging in mammals. The nature of this involvement; whether it is causative, predictive or a result of the state of senescence

ence, needs to be examined. Such knowledge can then be used to explore the age-associated diseases and the various diseases characterized by senility.

Proposed Course of Research:

We plan to utilize the tissue and cell lines established from Alzheimer pedigrees and other large pedigrees with familial disorders to identify genomic localization of viral sequences. The precise nature of the integrated viruses will be examined as well as potential disease associations. If this approach using the most current molecular biological techniques is promising, it will be extended to the investigation of other neuropsychiatric disorders.

The work will also examine the role of plasmids derived from the mitochondrial genome in the process of aging. Using tissues from young and aged individuals, the mitochondrial genome will be examined for evidence of amplification or excision of specific regions. The nuclear chromosomal DNA from tissue of aged persons and from fibroblast cell lines established from members of the Alzheimer's disease family will be screened for the integration of senescent plasmids. The occurrence of these events will be studied in their relationship with the symptoms of aging and the diseases associated with aging.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 00936-20 LGCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Homocystinuria: Methionine Metabolism in Mammals

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

S. H. Mudd Chief, Section on Alkaloid Biosynthesis

LGCB NIMH

COOPERATING UNITS (if any)

Dept. of Human Genetics, Yale University, New Haven, Conn.
Amino Acid Lab., Massachusetts General Hospital, Boston, Mass.

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Alkaloid Biosynthesis

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1

PROFESSIONAL:

1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

An international questionnaire survey is underway, aimed at obtaining standardized information to provide insight into many presently unanswered questions as to clinical features of homocystinuria due to cystathionine β -synthase deficiency and the effects of various therapies now in use for this condition. Data collection is now virtually complete. Information upon more than 630 patients has been received and entered into computer format. Analyses of the data are now underway. Although final results are not yet available, it is certain that the study will accomplish its chief aims: to provide base-line data for untreated patients with respect to mental retardation, thromboembolic episodes, lens dislocation, survival, and appearance of osteoporosis. These data will provide the necessary means to evaluate therapies as data accumulate in the future.

Other Principal Investigators:

F. Skovby	Postdoctoral Fellow, Dept. of Human Genetics, Yale University, New Haven, Conn., Visiting Associate, NIH Visiting Program.	LGCB, NIMH
H. L. Levy	Assist. Prof. of Neurology and Assist. Prof. of Pediatrics, Amino Acid Lab., Massachusetts General Hospital, Boston, Mass.	

Project Description:

Within the general framework of investigating methionine metabolism in mammals, with emphasis on the human genetic diseases which result from abnormalities affecting this area of metabolism, efforts this year have focussed upon a questionnaire survey of patients with homocystinuria due to cystathionine β -synthase deficiency. The purpose of the present survey is to gather sufficient information in a standardized format so that some of the major uncertainties about the natural history of this disease, about the role of genetic heterogeneity, and concerning the use and effectiveness of various therapies may be assessed.

The reasons which led us to conduct this survey, and the general design of the survey and the questionnaire were described in detail in last year's report upon this project, and need not be reiterated here.

Data collection for this survey was begun in February, 1982, and is now virtually complete, although occasional reports are still being received. More than 200 physicians have reported upon more than 530 patients. To this sample have been added data upon 98 patients reported in the literature upon whom updated reports were not received but who can be identified as not overlapping with patients already in the sample. The total (more than 628 patients) is far greater than the number of patients with cystathionine β -synthase deficiency of whom we knew when data collection began.

The data have been entered into computer format and development of suitable programs for analyses has commenced. Results are still preliminary and incomplete, but the following major conclusions appear to be justified:

(a) The available data will provide base-line curves for untreated patients for dislocation of optic lenses, thromboembolic events, survival, and development of osteoporosis, each as a function of patient age. As experience with treated patients accumulates, these curves will permit calculation of the expected rates of any of these end-points if the patients had been untreated, and therefore permit assessment of the impact of therapy.

(b) B₆-responsive patients have had fewer thromboembolic events during treatment with pyridoxine than would have been expected had they not been treated.

(c) The mean IQ of B₆-responsive patients is about 78, significantly higher than that of B₆-non-responsive patients (55), although extremely wide variations and overlap occur among both groups. The lowered IQ's are manifest from early ages (1-2 years). B₆-non-responsive patients detected by new-born screening and

treated with methionine restriction from early ages have virtually normal IQ's, and these do not deteriorate markedly as a function of age (the oldest members of this subgroup are now 10-12 years).

(d) Among late-detected patients classified for B₆-responsiveness, almost equal numbers are responsive as are non-responsive (229:230). Only 66 are intermediate in response. Among patients detected by newborn screening, 42 were B₆-non-responsive while only 7 were B₆-responsive. Responsive patients may be being missed by present newborn screening programs.

(e) There is a significant difference in the rate of lens dislocation between untreated B₆-responsive patients (50% dislocation at 9-10 years) and untreated B₆-non-responsive patients (50% dislocation by 5-6 years).

Eventually almost all patients develop dislocated lenses. The effect of therapy upon lens dislocation is difficult to evaluate based on present evidence (a) because a high proportion of late-detected patients have dislocated lenses at the time of ascertainment and (b) patients detected by newborn screening and started upon therapy with intact lenses, as a group are still too young and too few to have the statistical expectation of developing many lens dislocations. Nevertheless, the curves for lens dislocations resulting from the present study provide the basis for further evaluation of therapy as more data become available.

Ongoing analyses should permit similar evaluations of survival curves and of the appearance of osteoporosis.

Significance to Biomedical Research and the Program of the Institute:

The emphasis in this project has been upon human genetic diseases due to defects in the metabolism of sulfur-containing compounds. Many of these diseases lead to mental retardation; understanding their etiology and pathophysiology may be expected to help in prevention of such retardation and of other serious manifestations. Work on inborn errors has repeatedly been shown to illuminate normal human biochemistry and enzymology, and study of the diseases in question have likewise proven useful in furthering understanding of normal human metabolism.

Proposed Course of Research:

To complete this project, it is necessary only to develop a few more computer programs to permit analyses of survival and development of osteoporosis. Since they are essentially variations upon the approach already used to analyze lens dislocations and thromboembolic events, no major difficulties are foreseen. Once all programs have been developed, late reports will be incorporated into the data base used for analyses, and final runs will be made of all programs. Preparation of the final manuscript can then be carried out. It is hoped this entire process will be substantially completed within the next six months.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00939-03 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Enzymes of Methionine Biosynthesis in Higher Plants		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) G. A. Thompson Senior Staff Fellow LGCB NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Alkaloid Biosynthesis		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 0.33	PROFESSIONAL: 0.33	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Cystathionine γ -synthase from <u>Lemna paucicostata</u> has been partially purified by ammonium sulfate precipitation, gel-filtration upon Sephacryl G-300, and DEAE column chromatography. A 140-fold purification was achieved with excellent recovery of activity. The enzyme was physically separated from threonine synthase. The purified enzyme retains its capacity to utilize HS^- in place of cysteine, and can utilize also several <u>O-acylhomoserine</u> derivatives in place of O-phosphohomoserine. One-dimensional SDS-PAGE studies of the most purified cystathionine γ -synthase reveal that 10-15 protein bands are still present. The band representing cystathionine γ -synthase has not as yet been unequivocally identified.		

Other Principal Investigators:

J. Giovanelli	Research Chemist	LGCB NIMH
A. H. Datko	Botanist	LGCB NIMH
S. H. Mudd	Chief, Section on Alkaloid Biosynthesis	LGCB NIMH

Project Description:

A major objective of research in this laboratory is to elucidate the mechanism(s) of control of methionine biosynthesis in higher plants.

We have now demonstrated unequivocally that methionine down-regulates the flux of cysteine through the reaction catalyzed by cystathionine synthase. Part of this down-regulation is due to a decrease in the specific activity of cystathionine γ -synthase activity brought about by exogenous methionine. To gain further understanding of cystathionine γ -synthase, we have initiated purification of this enzyme. Ammonium sulfate precipitation, gel-filtration upon Sephacryl G-300, and DEAE column chromatography led to a 140-fold purification with excellent recovery of activity. The purified enzyme retains its capacity to utilize HS^- in place of cysteine, confirming our earlier evidence that O-phosphohomoserine sulfhydrylase and cystathionine γ -synthase activities are properties of a single enzyme. The purified enzyme can utilize also several O-acylhomoserine derivatives in place of O-phosphohomoserine, suggesting that the capacity of crude extracts to utilize these substrates is dependent only upon cystathionine γ -synthase and not upon additional enzymes. SDS-PAGE studies of the most purified cystathionine γ -synthase reveal that 10-15 protein bands are still present. The band representing cystathionine γ -synthase can not as yet be unequivocally identified. Considerable effort was expended in two-dimensional analyses of the same preparations, with and without specific inactivation of the cystathionine γ -synthase by propargylglycine, but the enzyme could still not be unequivocally identified.

Significance to Biomedical Research and the Program of the Institute:

This project is part of our general program to investigate the aspartate biosynthetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants," by Dr. Giovanelli.

Proposed Course of Research:

Unfortunately work on this project has been interrupted by Dr. Thompson's departure and our inability to replace him for budgetary reasons. Further purification of cystathionine γ -synthase would be most valuable in opening up several areas:

(a) Unequivocal identification of the enzyme on one- or two-dimensional gels. Once this has been achieved, it should be possible to gain insight into the molecular mechanisms by which methionine (or one of its products: ? AdoMet) regulates this enzyme. A useful maneuver to approach this question would also be to purify the residual enzyme from plants down-regulated by methionine.

(b) Production of antibody to cystathionine γ -synthase. Availability of antibody would permit studies of initial rates of synthesis and of turnover under various (regulatory) physiological regimens. It would also permit purification of messenger RNA for cystathionine γ -synthase from nascent ribosomes, a step which might ultimately furnish a c-DNA probe for cystathionine γ -synthase. Such a probe would be essential if cloning studies of cystathionine γ -synthase are to be carried out.

(c) Purified cystathionine γ -synthase could also profitably be studied with respect to its catalytic mechanism and how it is inactivated by PAG. Our results to date prove the Lemna enzyme is rather different in these respects than bacterial cystathionine γ -synthases which have been studied.

(d) Other important questions relate to the portions of cystathionine γ -synthase which are relatively resistant to PAG inactivation and/or to methionine down-regulation. Are these different molecular forms, or are they the consequence of sequestration of enzyme in different subcellular compartments? Purification of the PAG-resistant fraction and the enzyme remaining after methionine down-regulation could clarify these questions.

Which, if any, of these studies are actually undertaken is clearly dependent upon the availability of suitable personnel.

Publications:

Thompson, G.A., Datko, A.H., and Mudd, S.H.: Methionine synthesis in Lemna: Inhibition of cystathionine γ -synthase by propargylglycine. Plant Physiol. 70: 1347-1352, 1982.

Thompson, G.A., Datko, A.H., and Mudd, S.H.: Adaptation of Lemna paucicostata to sublethal methionine deprivation. Plant Physiol. 71: 241-247, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00940-03 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Methionine Biosynthesis in Higher Plants		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) A. H. Datko Botanist LGCB NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Alkaloid Biosynthesis		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.25	PROFESSIONAL: 1.25	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The low K_m system for sulfate uptake in <u>Lemna</u> is dramatically down-regulated by prior growth in <u>cystine</u> or <u>high concentrations of sulfate</u>. To look for possible site(s) of regulation, measurement was made of the steady-state concentrations of S-containing compounds in <u>Lemna</u> grown in concentrations of sulfate or cystine which down-regulate the uptake system to about the same extent. The results indicate that down-regulation is mediated by inorganic sulfate (or a product of its metabolism) in the case of growth in sulfate, but by cyst(e)ine (or a product of its metabolism) in the case of growth in cystine. In neither case is the product <u>methionine</u>. The apparent down-regulation of the low K_m system by about 40% by growth in methionine was shown to be a nonspecific effect. Properties of <u>Lemna</u> uptake systems for neutral amino acids, basic amino acids, purines, <u>choline</u>, <u>ethanolamine</u>, <u>aldohexoses</u>, and <u>urea</u> were determined. Measurement of kinetic constants and cross-competition experiments indicate that each is a separate system and that each has <u>high affinity</u> for the transported molecule. <u>Structural specificity</u> of the systems showed a range from broad specificity (i.e., any neutral L-α-amino acid; any aldohexose), to very strict structural requirements (i.e., choline; ethanolamine; urea). </p>		
(897)		

Other Principal Investigators:

S. H. Mudd Chief, Section on Alkaloid Biosynthesis

LGCB NIMH

Project Description:

A major aim of this laboratory is to elucidate the mechanisms in higher plants which serve to regulate the biosynthesis of the amino acids derived from the aspartate pathway with particular emphasis on the sulfur amino acid methionine. For methionine biosynthesis, a second pathway, that of sulfate uptake and reduction, must be integrated with the aspartate pathway. Sulfate uptake in Lemna is mediated almost entirely by a low K_m (high affinity) system at the concentrations of sulfate used in our standard growth medium, and this system is down-regulated when the plants are grown in cystine or unusually high concentrations of sulfate. A "high K_m " (low affinity) system contributes to sulfate uptake at much higher concentrations of external sulfate and this system is not down-regulated when the plants are grown in cystine or high concentrations of sulfate. This, along with other properties, suggested that the high K_m system may largely represent diffusion-mediated uptake.

Further observations on sulfate uptake in the past year have shown:

(1) The apparent down regulation of the low K_m system observed after plants were grown in methionine resulted from non-specific effects on uptake. When such effects are taken into account, growth in methionine does not affect either the "high" or low K_m sulfate uptake systems.

(2) A search for metabolites which mediate regulation of the low K_m system was initiated. Lemna was grown in $^{35}\text{SO}_4^{2-}$, ^{35}S -cystine, or ^{35}S -cystine and $^{35}\text{SO}_4^{2-}$ of the same specific radioactivity. For comparison, plants were grown in ^{34}S -methionine or ^{35}S -methionine and $^{35}\text{SO}_4^{2-}$ of the same specific radioactivity. Growth was carried out at a range of concentrations of sulfate, cystine or methionine for a sufficient length of time to assure isotopic steady-state of labeling was attained. The steady-state concentrations of the S-containing compounds of the plants was estimated by measuring the amount of ^{35}S -contained in each. Protein S was unaffected by the various growth conditions. At 10 mM sulfate where the low K_m uptake system is down-regulated to about 7% of control uptake, the inorganic sulfate pool was increased 4-fold in comparison with inorganic sulfate content in control plants, while soluble cyst(e)ine and methionine were unchanged. At 14 μM cystine where the low K_m uptake system was down-regulated to about 17% of control uptake, inorganic sulfate and soluble methionine were not increased in comparison with control values, while the soluble cyst(e)ine was increased 6-fold. At 2 μM methionine, where the low K_m system was not down-regulated there was a 300-fold increase in soluble methionine but the concentration of inorganic sulfate was not affected, and soluble cyst(e)ine was increased 3-fold. These results suggest that down-regulation brought about by growth in sulfate is mediated by sulfate or a product of its metabolism (which is not methionine) and down-regulation brought about by growth in cystine is mediated by cyst(e)ine or a product of its metabolism (which is not methionine). Conclusions on possible site(s) of regulation must, however, take into account the possibility that the critical metabolites are compartmented, so that further experiments will be necessary in order to

firmly establish these site(s). Such studies may also shed insight on the surprising result found with growth in methionine, where there was no down-regulation of sulfate uptake but an increase in soluble cyst(e)ine sufficient to down regulate uptake had it been brought about by cystine feeding.

(3) Several other interesting observations emerged from the analyses of the plant samples described in (2) above. Among these are:

(a) For growth in sulfate up to 10 mM, no component other than inorganic sulfate was significantly affected. It has been suggested that such a striking constancy of sulfate reduction in the face of greatly varied external sulfate is the result of close regulation of ATP sulfurylase, the first enzyme in sulfate reduction.

(b) Metabolism of the S in cystine in the direction cysteine \rightarrow SO_4^{2-} was extensive. Up to 25% of the ^{35}S in the cystine taken up appeared in inorganic sulfate. This represents a minimal estimate of cysteine desulfhydrase since it does not include ^{35}S which was metabolized to sulfide and subsequently organified, rather than appearing as sulfate.

(c) In contrast to the metabolism of cystine, essentially no ^{35}S derived from methionine appeared in inorganic sulfate, glutathione, or cysteine. We previously concluded, from inhibitor studies and substrate specificity of crude preparations of β -cystathionase, that transsulfuration in the direction of homocysteine to cysteine does not occur in higher plants at a rate sufficient to meet the plant's need for cysteine. A limit can now be set, based on present results, of less than 0.4% of the S in methionine being metabolized in the direction of homocysteine \rightarrow cysteine.

(d) For plants grown in 2 μM methionine, there was an increase in the steady-state concentrations of S-adenosylmethionine (AdoMet) and S-methylmethionine (S-MeMet). The concentration of AdoMet was increased some 12 x compared to control values. The resulting tissue concentration (assuming uniform distribution in the plant) is in the range of that which markedly stimulates the activity of Lemna threonine synthase activity in vitro. The concentration of S-MeMet was increased some 35 x compared to control values. The physiological function S-MeMet serves is not known. Study of its metabolism in Lemna, where it is known to be taken up and where its formation can be markedly affected, may shed insight into its function.

Since few mutants of amino acid biosynthesis in plants have been found, studies of regulation of such pathways must utilize other approaches such as, for example, studying the metabolic fate of an exogenously supplied metabolite or introducing metabolic blocks by administration of specific inhibitors. In the latter case, growth of the plant can be made dependent on administration of the amino acid intermediates normally formed after the site of the metabolic block. Often such inhibitors are themselves amino acids, and simultaneous administration of inhibitor and rescuing amino acid can affect the uptake of both compounds. To provide a rational basis for the design of such experiments, we have studied the amino acid uptake systems in Lemna. The study has been extended to include the uptake of other compounds whose metabolism impinges on that of methionine or whose uptake is relevant to understanding the normal growth of Lemna. Competition experiments showed that each prototype compound was taken up by a different system. The

systems, and the prototype compounds studied, and their characteristics are:

- (1) Neutral amino acid: Prototype L-leucine, K_m , 13.1 μM ; V_{max} , 16.2 nmole/frond d. Specific for compounds which are L-isomers, neutral, and which have both carboxyl and α -amino acid groups.
- (2) Basic amino acid: Prototype L-arginine; K_m , 3.6 μM ; V_{max} , 4.9 nmoles/frond d.
- (3) Purine: Prototype adenine, K_m , 0.3 μM ; V_{max} , 0.98 nmoles/frond d. Free purine bases and nucleosides, but not nucleotides, are taken up. There appears not to be a system for uptake of pyrimidines by Lemna.
- (4) Choline: K_m , 4.8 μM ; V_{max} , 3.6 nmoles/frond d. Removal of methyl groups, modification of the alcohol group, or phosphorylation yields compounds which are less effective as competitors for choline uptake.
- (5) Ethanolamine: K_m , 3.4 μM ; V_{max} 1.8 nmoles/frond d. Addition of methyl groups or modification of alcohol group to carboxyl very markedly reduces ability to compete with ethanolamine uptake. Substitution of amine group with hydroxyl or sulfide groups, or phosphorylation, has somewhat less marked effect.
- (6) Hexose: Prototype D-glucose, K_m , 73.4 μM ; V_{max} 27.4 nmoles/frond d. Uptake is specific for aldohexoses.
- (7) Urea: K_m , 1.2 μM ; V_{max} 3.5 nmoles/frond d. Substitution onto amine group, or replacement of O with S or NH very markedly reduces ability to compete with urea uptake. Substitution of amine group with methyl group has somewhat less effect.

Significance to Biomedical Research and the Program of the Institute:

This project is part of our general program to investigate the aspartate biosynthetic pathway in higher plants. The general significance of this research has been set forth in the report on the "Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants," by Dr. Giovanelli.

Proposed Course of Research:

A major limitation in our comprehension of the workings of the aspartate biosynthetic pathway in higher plants is due to lack of knowledge about the subcellular localization of the enzymes of this pathway, and, for the biosynthesis of the sulfur amino acids, of the sulfate reduction pathway as well. We plan to initiate attempts to isolate mitochondria, chloroplasts, and microsomes from Lemna in suitable form so that the subcellular distribution of relevant enzymes can be determined. It is hoped that the subcellular localization of metabolites, particularly cysteine and sulfate, can also be determined. Methods are now available for digesting plant cell walls. The resulting protoplasts can be carefully broken to yield not only the organelles listed above, but also the relatively fragile vacuoles. Cyst(e)ine and/or inorganic sulfate may be present in large part in the vacuole, as is known for nitrate. The extent of correlation of such physical compartments with the metabolic pools demonstrated in Lemna for cyst(e)ine is not

known. This sort of information is necessary in order to clarify the interaction between sulfate and cystine in down-regulating the low K_m sulfate uptake.

A search will be made for inhibitors which interfere with the conversion of methionine to S-adenosylmethionine. The latter compound has been proposed to be a major effector in the control of methionine and threonine biosynthesis in plants. Several compounds known to inhibit S-adenosylmethionine synthesis and metabolism in other tissues will be examined for their effects on Lemna growth. Some of the compounds of interest are amino acids (i.e., cycloleucine, ethionine) while others are derivatives of adenine (i.e., deazaadenosine). Our understanding of the properties of the Lemna uptake systems for these types of compounds will provide a basis for proper design of the planned experiments.

Lastly, there is reason to believe, both on enzyme-mechanistic grounds and from the nutritional results, that propargylglycine and aminoethoxyvinylglycine (AVG) may be affecting sites other than those revealed by studies to date. AVG is known to also inhibit ACC synthase, the enzyme which converts S-adenosylmethionine to 1-aminocyclopropane-1-carboxylic acid, the immediate precursor of the plant hormone, ethylene. Experiments are planned to examine if this, or other pathways are inhibited by this compound.

Publications:

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00941-03 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical Genetics and Metabolic Disease		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) <div style="display: flex; justify-content: space-between;"> C. R. Merrill Senior Research Scientist LGCB NIMH </div>		
COOPERATING UNITS (if any) Laboratory of Clinical Science, NIMH; Division of Cancer Cause and Prevention and Laboratory of Viral Carcinogenesis, NCI; Laboratory of Clinical Science, NIAAA; and Laboratory of Developmental Neurobiology, NICHD		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Proteins		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIMH, Bethesda, Maryland 20205		
TOTAL MANYEARS: <div style="text-align: center;">1</div>	PROFESSIONAL: <div style="text-align: center;">1</div>	OTHER: <div style="text-align: center;">0</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The primary goal of this study has been to explore the application of new biochemical methodologies to <u>human genetic diseases</u> affecting the central nervous system. A concentrated effort has been made with the techniques of <u>high resolution two-dimensional electrophoresis (2DE) autoradiography</u> and <u>ultra-sensitive silver staining</u>. Initial studies involved a disorder with a known biochemical basis, the <u>Lesch-Nyhan syndrome</u>. Currently, we are investigating a disease with a known genetic basis, but no known trait specific markers, <u>Huntington's disease</u>, and a disease with a suspected genetic basis, <u>familial Alzheimer's disease</u>. Large <u>multigenerational pedigrees</u> have been utilized. These pedigrees serve a dual role, they permit a greater chance to establish <u>trait specific markers</u> than smaller families, and they also serve as a resource in the development of a human <u>genome map</u> based on <u>polymorphic markers</u>. The establishment of a human genome map based on <u>polymorphic marker loci</u> will be an invaluable aid in investigations of any disease which contains a genetic component. The protein polymorphisms identified in this study will complement the <u>DNA polymorphisms</u> which are being identified in other laboratories. We have established cell lines from the individuals in the pedigrees under investigation. These cells are permitting investigations of additional paradigms for these diseases, such as the possible role for viral genes, which have been integrated into the human genome. </p>		

Other Investigators:

M. H. Ebert	Clinical Director	NIMH
D. Goldman	Chief, Unit on Molecular Genetics	LCS NIAAA
M. Miller	Biochemist	DCCP NCI
D. Jacobowitz	Associate Director, Laboratory of Clinical Science	LCS NIMH
S. O'Brien	Chief, Section on Genetics	LVC NCI
W. S. Rasband	Computer Systems Analyst	IRP NIMH
D. Klein	Research Physiologist	LDN NICHHD
W. Haydorn	Neurochemist	LCS NIMH
M. Harrington	Visiting Fellow	LCS NIMH

Project Description:

In an effort to extend our perception of the individual at the molecular level, we are exploring the applications of new biochemical methodologies. We have concentrated our effort on the technique of two-dimensional electrophoresis (2DE). This is a technique which permits the separation, identification and measurement of several thousand gene products which can be synthesized by any cell type. The use of such technology will allow increasingly accurate recognition, classification, understanding and treatment of pathology. There are four major areas in which a contribution to human medical genetics will be made: 1) the identification of primary mutations in genetic diseases; 2) the study of molecular alterations in inborn errors which may provide disease-specific and disease-associated markers; 3) observations of metabolic perturbations which will lead to a better understanding of normal metabolism and to the elucidation of metabolic patterns and pathways of diseases; and 4) the identification of protein polymorphisms and use of these polymorphisms in gene mapping.

Because genetic variants with metabolic consequences usually alter the quantity or quality of proteins, identification of alterations in protein quantity, composition, functional activity, localization, immunological determinants, or electrophoretic mobility have all contributed to our understanding of particular genetic diseases. In addition, these methods have helped reveal extensive, apparently selection-neutral, protein genetic variation. An unknown fraction of protein genetic variants confer gross structural and phenotypic variation and are the biochemical basis of phenotypic individuality. These are the genetic factors which help define us as individuals both in health and in disease.

Two-dimensional electrophoresis extends the capacity for detecting and studying the extent mechanisms and consequences of genetic variation, chiefly because it enables more than 1000 proteins to be visualized from a single cell type and can be applied with little modification to different cells and tissue fluids and their subfractions. Sensitive visualization methods, such as the silver stains developed in this laboratory allow largely indiscriminate detection so that the high resolving power of 2DE can be fully exploited. Discriminate detection, as with radiolabeling of phosphorylated proteins or detection with specific antibody is also useful. The large number of proteins resolved and the ready-made two-dimensional matrix make possible the systematized mapping and cross-correlation of new data, especially when 2DE is combined with the computer-assisted measurement and

data reduction, systems we have participated in developing.

Detection of Protein Polymorphism

Individual variation has both genetic and environmental origins. Genetic and environmental variation are manifested grossly or only on the molecular level, but all genetic phenotypic variation is determined by molecular variation and is most precisely studied at the molecular level. The study of the subtle molecular differences will lead to the dissection and understanding of human individuality. A category of normal genetic variation is the polymorphism. Polymorphisms are normal genetic phenotypic variants found with an allelic frequency of greater than 0.02. Although usually thought of as selectively neutral, an unknown fraction produce subtle metabolic and other phenotypic effects. Protein profiling by 2DE may help uncover which do so.

Of more immediate interest is the role molecular polymorphisms have to play for linkage analysis and for constructing a map of the human genome. For *Drosophila*, an effective genetic map was pieced together by Morgan and his students by 1926. This map was based solely on external characteristics and omitted only the Y chromosome. However, the process of mapping the human genome has lagged because of its greater size, the long species generation time and the fact that crosses cannot be arranged for experimental purposes and mutants cannot be deliberately induced in our society. However, the availability of molecular polymorphisms, in combination with linkage analysis and the use of somatic cell genetics, has now brought this goal within reach. Molecular polymorphisms which are generally useful for assigning new loci by linkage analysis are ones whose frequency is great enough that there is a reasonable likelihood that they will be present in a family under study.

Currently, less than 50 commonly polymorphic loci are available, including allozyme polymorphisms detectable by one-dimensional electrophoresis, serological antigen loci and DNA restriction fragment length polymorphisms (RFLPs). Discovery of new polymorphisms results in the probability that some additional fractions of the genome will be covered for genetic linkage studies. Currently, approximately 26% of the human genome is covered by linkage studies at a recombination fraction of 10%. Utilization of the polymorphisms available by two-dimensional electrophoresis will greatly increase the fraction covered if it can be shown that the polymorphisms being discovered are new ones. In a computer-assisted analysis of 28 individuals we have found 35 polymorphisms among 354 proteins surveyed in human lymphocytes, and fibroblasts.

In each case, the polymorphism is detected by a charge shift. In a general fashion, the extent of this shift in charge was found to vary inversely with molecular weight. Within the pH range examined, 2DE is thus capable of detecting almost all of the allozyme variants detected by one-dimensional electrophoresis because these variants generally involve charge shifts.

In addition to the shift in pI, there is often a slight shift in apparent molecular mass. This shift in SDS polyacrylamide gel mobility may be caused by altered conformation of the denatured protein in the second dimension gel and has been frequently observed for charge-substituted variants. Some recently described serum

polymorphisms showed a shift in apparent molecular mass but no shift in charge. Such variants could involve charge-neutral amino acid substitutions which result in a conformational change or which alter post-translational processing.

Gene dosage for 2DE protein polymorphisms has generally been observed when it has been looked for quantitatively. We have been able to demonstrate a gene dosage in each of the polymorphisms identified in our studies.

In the area of human population genetics, 2DE has enabled us to greatly expand the number of loci which can be assessed for variability. The over-all level of genetic variability in a population is expressed as average heterozygosity. Average heterozygosity is computed by summing the heterozygosity observed for each locus scored and dividing by the total number of loci scored. The average heterozygosity detectable by allozyme electrophoresis was 6.3% for man. We found an average heterozygosity of 2.4% for the 186 proteins surveyed in our human lymphocyte samples.

Although considerable progress has been made in the art of detecting 2DE polymorphisms, extensive family studies are just now beginning.

Protein Mutation by Two-Dimensional Electrophoresis

Discovery of new protein mutations and primary protein abnormalities in genetic disease by 2DE is an exciting prospect because no other technique allows so many gene products to be surveyed.

Some of the first clinical studies of inborn errors of metabolism involving abnormal behavioral patterns were done on the Lesch-Nyhan syndrome. The Lesch-Nyhan syndrome is an X-linked recessive trait due to a deficiency of hypoxanthineguanine phosphoribosyl transferase (HPRT) activity and results in spasticity, hyperuricemia and self-mutilation. Because HPRT activity was often undetectable and because some antibodies prepared against normal HPRT did not recognize mutant HPRTs, it was hypothesized that most of these patients were totally deficient in the protein. The mutations responsible for total absence of a protein could be mutations in a control protein or mutations at the structural locus which resulted in either a failure in transcription or early termination of translation (amber mutation). In E. coli, we successfully employed 2DE to demonstrate amber mutations in three enzymes of the galactose metabolic pathway: uridine diphosphate galactose 4-epimerase, galactokinase and galactose 1-phosphate uridylyl transferase. However, in the Lesch-Nyhan syndrome, we resolved, visualized and measured HPRT protein in each of three Lesch-Nyhan patients despite the virtual absence of enzyme activity and virtual absence of immunoprecipitable HPRT. In each case, there was only a slight reduction in HPRT protein on 2DE gels.

The detection of primary mutation remains the most difficult task in the investigation of genetic diseases. The major reasons for this are as follows:

1. Two-dimensional electrophoresis probably surveys only a fraction of the total cellular proteins. Based on the apparent number of mRNA species, the number of active cellular structural genes in somatic tissues is between 10,000 and 20,000, and the number is perhaps somewhat higher in brain, for which the data ranges from

12,000 to 70,000. If the mutation involves a minor polypeptide or one which is not visualized, it is likely to be missed. To overcome this difficulty, one may study tissues and subcellular fractions which are more likely to contain the protein whose mutation is responsible for the disease.

2. Even if the mutant protein is detectable, it may not show a charge alteration and it may be present in nearly normal concentration although its functional level is greatly reduced, as occurred in our Lesch-Nyhan study. One way of overcoming this difficulty is to study a sufficient number of subjects with a disease so that genetic heterogeneity will make likely the study of some individuals who manifest a charge mutation.

3. Charge variants discovered may be coincidental polymorphisms and must be proven to be otherwise.

4. Protein alterations not detected as charge variants are likely to be secondary alterations as described below. Such proteins can be useful as trait- or state-specific molecular markers and may give clues to pathogenesis.

Although detection of the specific mutation in a disease of unknown molecular origin is still unlikely, 2DE is far superior to any other method for this purpose. Two-dimensional electrophoresis also holds considerable promise for detecting the effects of mutagens, both in vitro and in the whole organism.

Characteristic Patterns of Polypeptide Modulation

Genetic traits and biological states may be associated with characteristic patterns of protein modulations. Some diseases which are phenocopies on a gross level may reveal trait-specific molecular markers due to secondary effects of the primary mutation.

The effects we are concerned with detecting are due to feedback control mechanisms, post-translational modification, altered protein stabilities or disrupted compartmentation. Patterns of protein modulation can be state-specific, or associated, or trait-specific of associated. The development of a correlative catalog of protein modulations in different diseases and will greatly assist in defining the specificity of protein modulations or patterns of modulations and will help dissect pathogenic and normal processes.

Patterns of state-associated protein modulations have been quantitatively characterized by exposing cells or tissues to a variety of treatments. These include heat shock, nerve growth factor, dibutyryl cAMP, calcium and thyroid hormone. Proteins have also been shown to vary with the cell cycle and after neoplastic transformation. In most cases, none of the observed modulations can be said to be state-specific because few treatments have been examined and because the identity and biology of modulation of most of the altered polypeptides is not understood.

Trait-associated protein alterations have now been identified in a number of human diseases. Merrill et al. found eleven significant quantitative alterations in which the quantitative alteration was greater than two-fold, among 400 lympho-

cyte protein surveys in patients with the Lesch-Nyhan syndrome. These differences were present in the patterns of all Lesch-Nyhan patients examined and were not found in any normal individuals. Secondary protein modulations have also been found in alterations of chromosome number, as in Trisomy 21. Proteins from aneuploid individuals may also display increased or decreased quantities which are secondary to gene dosage. In this context, such alterations might also be classified as trait-associated modulations. In this way, cytoplasmic superoxide dismutase (SOD-1) which is known to map to Chromosome 21 and which shows enzyme activity proportional to the number of copies of Chromosome 21, shows a density proportional to gene dosage when examined on 2DE gels of cells mono-, di- and trisomic for Chromosome 21. It should be noted that altered copy number for one chromosome may alter expression of genes on other chromosomes. For instance, activities of several enzymes which map to chromosomes other than 21 have been shown to be elevated in Down's syndrome. Nevertheless, using this approach, we have tentatively mapped two proteins, SOD-1 and an unknown 2DE protein, to Chromosome 21.

Studies of the tissue which are primarily involved in a disease process have usually yielded extensive differences when compared to the tissue in the normal state. These differences are at times due to cell death and altered cellular composition of the tissue. Brains of patients with Huntington's disease, Joseph's disease and multiple sclerosis show extensive protein alterations but most of these are secondary to gliosis. When peripheral fibroblasts and lymphocytes have been examined in Huntington's disease, no polypeptide alterations were detected. In our survey, although more than 300 proteins were quantitatively analyzed in 13 patients and 15 controls and individuals at risk for Huntington's disease, no abnormalities were observed in the patterns examined.

Significance to Biomedical Research and the Program of the Institute:

Clinical studies utilizing 2DE may contribute knowledge on polymorphism and linkage of polymorphic loci to disease, mutations (both new and disease-associated) with normal processes and disease. In the area of protein polymorphism, 35 human polymorphisms, including 24 which may be new ones, have been described and better estimates of human protein heterozygosity have been provided. For primary mutations, 2DE has been successfully used to identify charge-shift and amber mutations. For secondary protein modulations, patterns of these have been demonstrated in a number of genetic diseases. The specificity of these alterations as markers will be established as additional diseases and metabolic alterations are studied. The prospect of correlating the information that is being accumulated is good owing to the suitability of the high resolution 2DE matrix to cataloging and the application of the method to a growing variety of questions. Many diseases of the central nervous system can be diagnosed only by their symptoms. Evidence of genetic involvement has been obtained by family studies in some of these diseases and biochemical abnormalities have been reported in a few. The techniques developed in this project will permit surveys for trait-specific and state-specific disease protein markers on a scale that would not have been possible in the past proposed course of research.

Proposed Course of Research:

We would like to continue these studies concentrating on a known genetic basis, but with no known trait specific markers, Huntington's disease and a disease with a suspected genetic basis, familial Alzheimer's disease. In these studies, we have chosen to utilize large multigenerational pedigrees. The use of such pedigrees will serve a dual role: they will permit a greater chance of establishing a definitive linkage with disease markers, and they will also serve as a resource for the development of a human genome map. Such a map will be an invaluable aid in investigations of all diseases with genetic components. We would also like to establish cell lines from these pedigrees. These cell lines will permit investigations of additional paradigms for these diseases, such as the role of viral genes which are integrated in the human genome.

Publications:

Van Keuren, M.L., Goldman, D., and Merrill, C.R.: Protein variations associated with Down's syndrome, Chromosome 21 and Alzheimer's disease. In Sinex, F.M., and Merrill, C.R. (Eds.): Annals of the New York Academy of Sciences, New York, New York Academy of Sciences, 1982, Vol. 396, pp. 55-67.

Merrill, C.R., Goldman, D., Van Keuren, M.L., and Ebert, M.H.: Molecular probes for human genetic diseases by two-dimensional protein electrophoresis and silver staining. In Electrophoresis '82. Berlin, Walter de Gruyter and Co., 1983, pp. 327-342.

Van Keuren, M.L., Merrill, C.R., and Goldman, D.: Proteins affected by Chromosome 21 and aging in vitro. In Celis, J.E., and Bravo, R. (Eds.): Gene Expression in Normal and Transformed Cells. New York, Plenum, 1983, pp. 349-378.

Myers, R.H., Goldman, D., Bird, E.D., Sax, D.S., Merrill, C.R., Schoenfeld, M., and Wolf, P.A.: Maternal transmissions in Huntington's disease. Lancet, in press.

Merrill, C.R., Goldman, D., and Van Keuren, M.L.: Silver staining methods for PAGE. In Methods in Enzymology. New York, Academic Press, in press.

Merrill, C.R., and Goldman, D.: Detection of polypeptides in two-dimensional gels using silver staining. In Clinical Applications of Two-Dimensional Electrophoresis. New York, Academic Press, in press.

Goldman, D., and Merrill, C.R.: Two-dimensional electrophoresis for studies of inborn errors of metabolism. In Clinical Applications of Two-Dimensional Electrophoresis. New York, Academic Press, in press.

Merrill, C.R., Goldman, D., and Van Keuren, M.L.: Silver staining. In Methods in Enzymology. New York, Academic Press, in press.

Goldman, D., and Merrill, C.R.: Human lymphocyte polymorphisms detected by quantitative two-dimensional electrophoresis. Am. J. Hum. Genet., in press.

Van Keuren, M.L., Merrill, C.R., and Goldman, D.: Protein variations associated with in vitro aging of human fibroblasts and quantitative limits on the error catastrophe hypothesis. J. Gerontol., in press.

O'Brien, S.J., Wildt, D.E., Goldman, D., Merrill, C.R., and Bush, M.: The cheetah is depauperate in genetic variation. Science, in press.

Goldman, D., and Merrill, C. R.: Detection of human lymphocyte polymorphisms with quantitative two-dimensional electrophoresis. Proc. Natl. Acad. Sci. U. S. A., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 00942-02 LGCB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Biochemical Reactions in Mammalian Cell Chemotaxis

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

G. L. Cantoni Chief, Lab. Gen. Comp. Biochem.

LGCB NIMH

COOPERATING UNITS (if any)

Office of Biologics, NCDB

Laboratory of Molecular Biology, NCI

LAB/BRANCH

Laboratory of General and Comparative Biochemistry

SECTION

Section on Proteins

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

2

PROFESSIONAL:

1.5

OTHER:

0.5

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Chemotaxis by the RAW264 mouse macrophage cell line was inhibited by 3-deaza-adenosine but not by 3-deazaaristeromycin. Observations of the cells by time-lapse video photography indicated that cells treated with 3-deazaadenosine recognized the attractant and were motile, suggesting that treatment with 3-deazaadenosine inhibited signal processing. Determination of the change in intracellular levels of S-adenosylhomocysteine (AdoHcy) and 3-deazaadenosylhomocysteine (3-deaza-AdoHcy) showed that inhibition of chemotaxis was correlated with the increase in 3-deaza-AdoHcy, formed intracellularly by the utilization of 3-deazaadenosine as a substrate for AdoHcy hydrolase. The synthesis of phosphatidylcholine by stepwise methylation of phosphatidylethanolamine, the attractant-induced release of arachidonic acid, methylation of lysine and arginine residues in protein, and carboxymethylation were inhibited by both 3-deazaadenosine and 3-deazaaristeromycin, indicating that these reactions were not required for chemotaxis. In addition, cAMP levels were not significantly changed in cells incubated with 3-deazaadenosine or 3-deazaaristeromycin. However, the synthesis of a small number of proteins, separated by two-dimensional polyacrylamide gel electrophoresis, was susceptible to 3-deazaadenosine, but not to 3-deazaaristeromycin. Quantitation of the gels by computerized densitometry showed that the synthesis of approximately 10% of the proteins was inhibited by more than 50%. A correlation was found between inhibition of chemotaxis and inhibition of the synthesis of the same subset of proteins when other compounds were tested. These compounds also inhibited the synthesis of polyadenylated RNA, leading us to postulate that incubation of cells with 3-deazaadenosine inhibits a methylation reaction that is required for the formation of a functional mRNA coding for one or more proteins required for chemotaxis.

Other Principal Investigators:

R. R. Aksamit	Senior Staff Fellow	LGCB NIMH
P. S. Backlund, Jr.	Staff Fellow	LGCB NIMH

Other Investigators:

T. M. Caryk	Chemist	LGCB NIMH
L. Harvath	Staff Fellow	OB NCDB
M. Willingham	Chief, Ultrastructural Cytochemistry Section	LMB NCI
I. Pastan	Chief, Laboratory of Molecular Biology	LMB NCI

Project Description:

The important discovery in this laboratory that chemotaxis by a macrophage cell line is specifically inhibited by 3-deaza-AdoHcy has allowed us to assess the significance of certain biochemical reactions in macrophage chemotaxis. For these studies we chose the cloned RAW264 mouse macrophage cell line which is easily grown in tissue culture and is amenable to genetic manipulation. Other studies of leukocyte chemotaxis have had the disadvantage that the cells were heterogeneous with respect to cell type and cellular behavior. Chemotaxis by RAW264 cells is inhibited by 3-deaza-AdoHcy, formed by AdoHcy hydrolase when 3-deazaadenosine is administered to the cells. In addition to the accumulation of 3-deaza-AdoHcy, AdoHcy also accumulates due to inhibition of AdoHcy hydrolase. Fortunately, another inhibitor of AdoHcy hydrolase, 3-deazaaristeromycin, has been developed in this laboratory that inhibits AdoHcy hydrolase but does not function as a substrate for the enzyme. Administration of 3-deazaaristeromycin to the cells resulted in the accumulation of AdoHcy to levels that are higher than those achieved by administration of 3-deazaadenosine. Therefore, since 3-deazaaristeromycin had no effect on chemotaxis, it was possible to conclude that chemotaxis was specifically inhibited by 3-deaza-AdoHcy.

A search was initiated for a reaction that was inhibited in cells treated with 3-deazaadenosine and not inhibited in cells treated with 3-deazaaristeromycin. The synthesis of phosphatidylcholine by methylation of phosphatidylethanolamine, the release of arachidonic acid when cells are incubated with EAMS (endotoxin-activated mouse serum, an attractant for mouse macrophages), methylation of the lysine and arginine residues of protein, and protein carboxymethylation were all inhibited by both 3-deazaadenosine and 3-deazaaristeromycin. Therefore, these reactions do not have the necessary specificity and, although other laboratories have reported that several of these reactions are involved in leukocyte chemotaxis, they are probably not required for chemotaxis by RAW264 cells. In addition cAMP levels were not significantly changed in cells incubated with 3-deazaadenosine or 3-deazaaristeromycin.

A reaction was found that was inhibited when cells were incubated with 3-deazaadenosine but not with 3-deazaaristeromycin. The reaction is the inhibition of the synthesis of one or a small number of proteins, separated by two-dimensional polyacrylamide gel electrophoresis. Quantitation of the gels by computerized densitometry showed that the synthesis of approximately 10% of the proteins was

inhibited by more than 50%. The correlation of this reaction with chemotaxis was strengthened by the finding that other inhibitors of chemotaxis inhibited the synthesis of the same subset of proteins. These inhibitors are 3'-deoxyadenosine and erythro-9-(2-hydroxy-3-nonyl)-adenosine (EHNA) in the presence of adenosine and homocysteine. A common feature of the inhibitors of chemotaxis described above is that all of them can inhibit the synthesis of functional mRNA. In this regard, we have also found that inhibitors of protein synthesis and translation, such as cycloheximide, puromycin, and actinomycin D, inhibit chemotaxis.

We have proposed as a working hypothesis that treatment of RAW264 cells with 3-deazaadenosine, 3'-deoxyadenosine, and the combination of EHNA, adenosine and homocysteine inhibit the synthesis of functional mRNA coding for one or more chemotactic proteins. In support of this hypothesis, we have found that 3-deazaadenosine is a more potent inhibitor of polyadenylated RNA than 3-deazaaristeromycin.

One of our goals is to determine which biochemical reaction(s) in chemotaxis is inhibited by treatment of the cells with 3-deazaadenosine. As a first step toward this goal, we observed the motility of RAW264 cells by time-lapse video cinematography. RAW264 cells were motile and morphological changes occurred when the cells were exposed to attractant. Incubation of cells with 3-deazaadenosine, under conditions that inhibited chemotaxis by approximately 90%, did not noticeably affect motility or the response to attractant. These observations suggest that in cells treated with 3-deazaadenosine, signal processing after binding to the chemoreceptor is inhibited.

Significance to Biomedical Research and the Program of the Institute:

Mammalian cell chemotaxis is important in the development of the nervous system, inflammation and wound healing. Chemotaxis is also a behavior response at the cellular level. Studies of bacterial chemotaxis from the laboratories of Koshland and Adler have shown that bacteria have "memory" and adapt to their environment, and progress has been made in explaining these concepts in molecular terms. Since it has been repeatedly shown that the biochemical reactions of bacteria are fundamentally similar to those of higher organisms, it might be expected that the molecular reactions involved in memory and adaptation for bacteria may also occur in these processes in mammalian cells. The mammalian cell line model for chemotaxis that we have developed provides a mammalian system to test these reactions and to study signal transmission.

A large number of methylation reactions are known and are involved in the synthesis of DNA, RNA, proteins, lipids and neurotransmitters. Inhibitor studies have indicated that methylation reactions are involved in cell differentiation, virus replication, and chemotaxis. However, the association between a specific transmethylation and a physiological response has not been defined in a mammalian system. Such information would be valuable in the design of antiinflammatory and antiviral drugs.

Proposed Course of Research:

Future work will be directed toward verification of the hypothesis that 3-deaza-AdoHcy specifically inhibits the synthesis of functional mRNA coding for one or more chemotactic proteins, and toward the identification of biochemical reactions important in chemotaxis. These problems will be approached by a combination of biochemical and genetic techniques.

Publications:

Aksamit, R.R., Backlund, P.S., Jr., and Cantoni, G.L.: Chemotaxis and the synthesis of specific proteins are inhibited by 3-deazaadenosine and other adenosine analogs in a mouse macrophage cell line. J. Biol. Chem. 258: 20-23, 1983.

Aksamit, R.R., and Backlund, P.S., Jr.: Chemotaxis and methylation in a macrophage cell line. Surv. Immunol. Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00943-02 LGCB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pathways of Methionine and Threonine Metabolism and Their Control in Higher Plants		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J. Giovanelli Research Chemist LGCB NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of General and Comparative Biochemistry		
SECTION Section on Alkaloid Biosynthesis		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1	PROFESSIONAL: 1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Threonine synthase from <u>Lemna</u> was purified some 50-fold, and separated from cystathionine γ-synthase. Threonine synthase showed a high specificity for the carbon substrate (O-phosphohomoserine) and the nucleophile (hydroxyl ion); nucleophilic attack by hydroxyl ion was restricted to carbon 3 of O-phosphohomoserine and was stereospecific. No evidence was obtained for major feedback inhibition, repression or covalent modification of the enzyme by threonine and/or isoleucine. Preliminary experiments indicate that two unexpected properties may be of regulatory significance. The first is that threonine synthase activity in gel-filtered extracts was decreased approximately 50% by growth of plants with <u>methionine</u>, and increased in plants limited in methionine by growth with lysine + threonine. These observations suggest possible down-regulation of threonine synthase by excess methionine. The second unexpected property is that threonine synthase was inhibited (approx. 70%) by <u>AMP</u> (67 μM) or <u>P_i</u> (1 mM), while cystathionine γ-synthase was relatively insensitive to these compounds. Inhibitions comparable to those with AMP or P_i were not obtained with a variety of other nucleotides or anions (pyrophosphate, sulfate, chloride). These findings raise the possibility that the physiological concentrations of AMP (20 μM) and P_i (14 mM) in <u>Lemna</u> may inhibit the activity of threonine synthase relative to that of cystathionine γ-synthase, and thereby affect the balance of fluxes into the threonine and methionine biosynthetic branches.</p> <p>Preliminary results indicate that 5'-chloroadenosine (ChlAdo) is an effective inhibitor of the conversion of <u>methylthioadenosine</u> (MTA) to methionine. ChlAdo prevents MTA from relieving the severe growth inhibition caused by the combined presence of propargylglycine and lysine + threonine; the same concentration of ChlAdo alone had no effect on growth. These experiments suggest that conversion of MTA to methionine is probably not an absolute requirement for normal growth on <u>Lemna</u>, but becomes crucial when net synthesis of methionine via transsulfuration is inhibited e.g. by propargylglycine and lysine + threonine.</p>		

Other Investigators:

K. Veluthambi	Visiting Fellow	LGCB NIMH
S. H. Mudd	Chief, Section of Alkaloid Biosynthesis	LGCB NIMH
A. H. Datko	Botanist	LGCB NIMH
G. A. Thompson	Senior Staff Fellow	LGCB NIMH

Project Description:

Most of our efforts have been concentrated on the enzyme threonine synthase and its regulation. The chief results are as follows:

(a) Threonine synthase from Lemna was purified some 50-fold by ammonium sulfate fractionation, gel filtration on Sephacryl S-300, and ion exchange chromatography on DEAE-Sephacel. The gel filtration step separated threonine synthase (MW approx. 50,000) from cystathionine γ -synthase (MW approx. 250,000), which also uses O-phosphohomoserine as substrate and proceeds through a mechanism similar to that for threonine synthase.

(b) Threonine synthase showed a high specificity for the carbon substrate (O-phosphohomoserine) and the nucleophile (hydroxyl ion); nucleophilic attack by hydroxyl ion was restricted to carbon 3 of O-phosphohomoserine and was stereospecific. The high degree of substrate specificity of threonine synthase contrasts with that of cystathionine γ -synthase from Lemna which is active with a variety of homoserine esters besides O-phosphohomoserine, and other sulfur nucleophiles besides cysteine.

(c) Since threonine synthase catalyzes the first committing step in the threonine biosynthetic branch of the aspartate pathway, it is a likely candidate for feedback regulation. However, no evidence was obtained for major feedback inhibition, repression or covalent modification of the enzyme by threonine and/or isoleucine. Preliminary experiments indicate that two unexpected properties may be of regulatory significance. The first is that threonine synthase activity in gel-filtered extracts was decreased approximately 50% by growth of plants with methionine, and increased in plants limited in methionine by growth with lysine + threonine. These observations suggest possible down-regulation of threonine synthase by excess methionine. The second unexpected property is that threonine synthase was inhibited (approx. 70%) by AMP (67 μ M) or P_i (1 mM), while cystathionine γ -synthase was relatively insensitive to these compounds. Inhibitions comparable to those with AMP or P_i were not obtained with a variety of other nucleotides or anions (pyrophosphate, sulfate, chloride). At the physiological concentration of 14 mM P_i in Lemna, the ratio of activities of threonine synthase to cystathionine γ -synthase was reduced to approx. 20% of that for the uninhibited enzymes, and the additional presence of AMP at the physiological concentration of 20 μ M would be expected to reduce this ratio further. These findings raise the possibility that *in vivo* P_i and AMP may inhibit the activity of threonine synthase relative to that of cystathionine γ -synthase, and thereby affect the balance of fluxes into the threonine and methionine biosynthetic branches.

Our studies on the methionine biosynthetic branch have concentrated on finding an inhibitor of the conversion of methylthioadenosine (MTA) to methionine. Preliminary results indicate that 5'-chloroadenosine (ChlAdo) effectively inhibits this

conversion. Thus early studies on the metabolism of [methyl- ^{14}C]MTA by Lemna show that ChlAdo markedly inhibits incorporation of radioactivity into methionine and its products. ChlAdo prevents MTA from relieving the severe growth inhibition caused by the combined presence of propargylglycine and lysine + threonine; the same concentration of ChlAdo alone had no effect on growth. These experiments suggest that conversion of MTA to methionine is probably not an absolute requirement for normal growth on Lemna, but becomes crucial when net synthesis of methionine via transsulfuration is inhibited e.g. by propargylglycine and lysine + threonine.

Significance to Biomedical Research and the Program of the Institute:

The primary goal of this project is to elucidate the pathways for methionine and threonine metabolism, and their control, in higher plants, using Lemna as an experimental system. Methionine and threonine biosynthesis are closely related in plants, the two pathways branching at the common intermediate O-phosphohomoserine. There are now a number of indications that regulation of the two biosynthetic branches may also be interrelated. Our research on each of these two amino acids therefore continues to be directed along parallel and complementary lines. This project is significant to the research goals of the Institute since methionine and threonine are among the four most commonly limiting essential amino acids in the human diet. Deficiency of these amino acids (especially during early life) in protein-calorie malnutrition may be accompanied by irreversible retardation in mental development. Plant proteins provide the source of these two amino acids, almost entirely, either directly by ingestion of plant material, or indirectly through an animal intermediate. Many of the plant foods most used by man are deficient in one or both of the amino acids, methionine and threonine. An understanding of the patterns of control of the biosynthesis and metabolism of methionine and threonine will provide a rational basis for maximizing the production of these essential dietary components.

Proposed Course of Research:

- (1) The partially purified preparation of threonine synthase will be used to determine the mechanism of its inhibition by AMP and P_i , and the structural specificity and other properties of its allosteric stimulation by AdoMet.
- (2) Our preliminary experiments indicating that excess methionine may down-regulate threonine synthase raises the intriguing possibility that allosteric stimulation of threonine synthase by AdoMet may not be the only mechanism by which products of the methionine synthetic branch may cross-regulate the threonine synthetic branch. The extent to which excess methionine may down-regulate threonine synthase will therefore be determined, and the physiological significance of this effect and its relation to the down-regulation of cystathionine γ -synthase by methionine will be elucidated.
- (3) The physiological significance of AMP and P_i in regulating the balance of fluxes into the methionine and threonine biosynthetic branches will be examined with Lemna growing under conditions that produce either a surplus or deficiency of AMP or P_i .

(4) The extent to which the in vivo fluxes into the threonine biosynthetic branch is affected either by potential feedback regulators of threonine biosynthesis, or by cross-regulation from the methionine biosynthetic branch will be examined in labeling experiments with intact Lemna.

(5) We will continue our search for a relatively specific inhibitor of threonine synthase. Such an inhibitor would be extremely useful in assessing the physiological significance of this enzyme in controlling the flux into the threonine biosynthetic branch.

(6) On the basis of experiments with double labeled methionine, we have previously outlined the major pathways for methionine metabolism in Lemna, and made quantitative estimates of the fluxes through these pathways. We plan to define these pathways in more detail and quantitate the fluxes more precisely in experiments in which Lemna is incubated with methionine that is labeled specifically in the appropriate moiety. In addition, we will examine how major pathways for methionine metabolism such as transmethylation (and regeneration of the homocysteine moiety of methionine) and polyamine synthesis (and methionine thiomethyl recycling) are regulated, and how these regulatory processes are integrated.

(7) Studies on the cell-free synthesis of methionine from MTA, which are still at an early stage, will be continued in an effort to optimize the system, and to define the intermediates in the pathway. Of special interest is whether the pathway in plants proceeds via methylthioribose (as in bacteria) or methylthioribose-1-phosphate (as in animals).

(8) The site of action, mechanism and other properties of the inhibition by ChlAdo of the conversion of MTA to methionine will be determined. It is hoped that these studies will also help to elucidate intermediates in the multistep conversion of MTA to methionine.

Publications:

Giovanelli, J., Datko, A.H., Mudd, S.H., and Thompson, G.A.: In vivo metabolism of 5'-methylthioadenosine in Lemna. Plant Physiol. 71: 319-326, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00981-18 LNB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Detection and interpretation of mechanical changes in the nervous system.		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> Ichiji Tasaki, Chief, Laboratory of Neurobiology, NIMH		
COOPERATING UNITS <i>(if any)</i>		
LAB/BRANCH Laboratory of Neurobiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3	PROFESSIONAL: 2	OTHER: 1
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p> By extending the discovery of the phenomenon of <u>swelling in invertebrate nerve fibers</u> during <u>excitation</u>, we found that the <u>spinal ganglion</u> of the frog swells when the ganglion cells are excited by way of the dorsal root or the sciatic nerve. The time-course of the swelling is similar to that of the action potential of the ganglion cells and is considered to reflect a drastic change in the distribution of water molecules in and near the <u>neuronal membrane</u>. We found also that the spinal cord of the toad and of the frog swells (namely, expands laterally) and retracts (namely, shortens longitudinally) when a volley of impulses enters the cord via dorsal roots. The duration of this mechanical change is very long (about 100 msec or more) and is comparable to that of the <u>dorsal root potential</u>. A volley of impulses entering the cord antidromically via <u>ventral roots</u> produces only a small, brief contraction of the cord. We have examined the basic properties of these <u>mechanical changes</u> in the nervous system and interpreted the results obtained on the basis of our knowledge about the phenomenon of swelling of invertebrate nerve fibers. </p>		
(919)		

Project Description:

Others involved in this project are Kunihiro Iwasa, Senior Staff Fellow, NIMH, LNB and Paul M. Byrne, Biomedical Engineering Technician, NIMH, LNB.

Objectives:

Elucidation of the molecular basis of excitation processes in the nervous system by detecting and analyzing mechanical changes in nerve cells and fibers.

Methods Employed:

Invertebrate nerve fibers of the crab, lobster and squid were employed to study physicochemical bases of the phenomenon of swelling in nerve fibers. For detection and analyses of mechanical changes in the vertebrate central nervous system, isolated spinal cord preparations of the bullfrog and toad were used. Mechanoelectric transducers employed include (1) piezoceramic bender (purchased from Gulton Industries, Inc.); (2) small aluminum levers used in conjunction with a Fotonic sensor (Mechanical Technology, Inc.); and (3) polyvinylidene film (Kureha Chemical Co.).

Major Findings:(1) Swelling of ganglion cells during excitation.

We found that the spinal ganglion of the bullfrog swells when excited by invading nerve impulses. The time-course of this swelling was similar to that of the action potential of the nerve cells. The peak value of the swelling pressure was comparable to the value observed in invertebrate nerve fibers (namely, a few dyn/cm²). This mechanical change is considered to be analogous to the swelling of the squid giant during the action potential.

(2) Contraction of the spinal cord upon arrival of afferent impulses.

We found that a volley of impulses entering the toad spinal cord via large myelinated fibers in the lumbar dorsal roots evoked a contraction of the cord, which lasted for about 100 msec or more. A volley entering the cord antidromically via the ventral roots produced only a small, brief contraction. When two electric shocks were delivered to the same dorsal roots at a short interval, the contraction associated with the second shock was small; a period of about 1 sec was required for a full recovery. When two shocks were applied separately to two neighboring dorsal roots, the contraction associated with the second shock was partially or totally occluded. Electric polarization of the dorsal root fibers produced mechanical changes in the cord. The effects of magnesium salt, GABA, glutamate and several other neuropharmacological agents on the contractile process were investigated. The experimental findings suggests that the contractile process is directly related to the phenomenon of primary afferent depolarization.

(3) Elucidation of physiocochemical bases of the phenomenon of swelling in the nerve fiber.

The degree of slow swelling of crab nerve fibers in potassium-rich media was dependent on the anion species present in the external media. Similar anion dependence of swelling was observed during veratridine depolarization of nerve fibers. These findings were interpreted as indicating that cytoskeletal proteins play an important role in slow swelling.

The squid axon was found to act as a nearly ideal osmometer when it was immersed in hypertonic media. These axons did not swell appreciably when immersed in hypotonic media. After enzyme digestion of the layer of connective tissue, the axon became a roughly ideal osmometer.

By combining NMR (nuclear magnetic resonance) spectroscopy with the radio-isotope technique, we found that both Mn(II) and Co(II) are taken up by nerve fibers. We conclude from these findings that NMR spectroscopy used in combination with transition metal ions is an unreliable method for distinguishing intracellular water from extracellular water.

Scientific Significance and Relevance to Public Mental Health:

The phenomenon of swelling of nerve fibers and cells is directly related to changes in the interaction of membrane and cytoskeletal proteins with small ions and water molecules. There is little doubt that such changes in the degree and the type of interaction are at the base of the normal process of excitation and inhibition in the nervous system. We believe, therefore, that experimental studies along the aforementioned line led us to a better understanding of the normal function, as well as abnormal behavior, of the nervous system.

Proposed Course:

We have just started our investigation into the contractile processes in the central nervous system. We are planning to continue to examine these mechanical changes in relation to other physicochemical and electrophysiological events that take place in the spinal cord upon arrival of afferent nerve impulses. We are also planning to investigate various aspects of interactions between subaxolemmal proteins, small ions, and water.

Publications:

Iwasa, I., Joshi, Y.M., and Kwak, J.C.T.: The influence of polyion structure in colligative and transport properties of polyelectrolyte solutions. In Oosawa, F. (Ed.): Dynamic Aspects of Biopolyelectrolytes and Biomembranes. Tokyo, Japan, Kodansha, 1982, pp. 26-42.

Iwasa, K. and Tasaki, I.: Molecular events that underlie membrane excitation. J. Theoret. Biol. 99: 87-99, 1982

Iwasa, K.: Mean activity coefficient for polyelectrolyte in mixed aqueous solutions of simple electrolyte and polyelectrolyte. J. Chem. Phys. 77: 2078-2080, 1982.

Tasaki, I. and Iwasa, K.: Further studies of rapid mechanical changes in squid giant axon associated with action potential production. Jpn. J. Physiol. 32: 455-468, 1982.

Allen, R. D., Metuzals, J., Tasaki, I., Brady, S. T., and Gilbert, S. P.: Fast axonal transport in squid giant axon. Science 218: 1127-1129, 1982.

Tasaki, I. and Byrne, P. M.: Tetanic contraction of the crab nerve evoked by repetitive stimulation. Biochem. Biophys. Res. Commun. 106: 1435-1440, 1982.

Tasaki, I. and Iwasa, K.: Rapid mechanical changes in crab nerve and squid axon during action potentials. J. Physiol., (Paris), 77: 1055-1059.

Tasaki, I. and Byrne, P. M.: Swelling of frog dorsal root ganglion and spinal cord produced by afferent volley of impulses. Brain Res., in press.

Tasaki, I. and Iwasa, K.: Axolemma-ectoplasm complex and mechanical responses of the axon membrane. In: Chang, D., Tasaki, I., Adelman, W., and Leuchtag, R. (Eds.) Structure and Function in Excitable Cells. Plenum, in press.

Iwasa, K.: Mechanical changes. In: Watanabe, A. and Yamagishi, S. (Eds.): Sin Seiri-kagaku Taikei (Handbook of Physiology, New Series), Igaku-Shoin, Tokyo. in press.

Iwasa, K.: Anion-dependent swelling of crab nerve fibers during potassium and veratridine depolarization. Physiol. Chem. Phys., in press.

Iwasa, K. and Inubushi, T.: Co^{2+} and Mn^{2+} uptake by crab nerve fibers in resting state and potassium depolarization. Biochem. Biophys. Res. Comm., 111: 560-566, 1983.

Iwasa, K.: Osmotic properties of the squid giant axon and their modifications. Cell. Mol. Biol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 00983-05 LNB
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical studies on the mechanism of nerve excitation.		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jesse Baumgold, Research Chemist, Laboratory of Neurobiology, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurobiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2	PROFESSIONAL: 1	OTHER: 1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In nerve and in muscle, action potentials are mediated by specialized <u>mem-</u> <u>brane proteins</u> in the plasma membrane of these cells. Although the function of these proteins, usually referred to as <u>ion channels</u> or excitable sites, has been well characterized electrophysiologically, the biochemistry of these proteins has hardly been studied. The goal of this research project is to study the biochemis- try of one particular such protein, known as a <u>sodium channel</u> protein. This pro- tein binds several <u>neurotoxins</u>, including <u>saxitoxin</u> and <u>scorpion toxin</u>, which can be radioactively labeled and used as specific molecular probes. </p> <p> During the course of cellular differentiation, myoblasts and neuroblasts, the developmental precursors of muscle and nerve cells, gradually acquire the proteins needed for electrical excitability. By following the biochemical events related to the acquisition of these proteins, we found the following. Based on detailed neurotoxin binding studies in muscle cells, we found that ¹²⁵I-scorpion toxin bind- ing activity develops earlier, faster, and reaches mature levels well before ³H- saxitoxin binding activity. Since the electrophysiological development parallels the slower ³H-saxitoxin binding activity, we proposed a model for the development of this protein. The models predicts that this protein gets inserted into the plasma membrane in an immature, electrophysiologically inactive form, capable of binding only ¹²⁵I-scorpion toxin. During subsequent development, this protein undergoes a <u>post-translational modification</u> which renders it electrophysiologi- cally active and capable of binding both ¹²⁵I-scorpion and ³H-saxitoxin. </p> <p> We have solubilized and partially purified this protein from rat brain. By comparing the behavior of this protein from mature and immature rat brain on ion exchange and on lectin affinity columns, we found that the mature and immature forms are differentially glycosylated. This differential glycosylation may be the post-translation modification predicted by our hypothesis. We are currently further characterizing these differences and are raising <u>monoclonal antibodies</u> to to the two different forms of this protein, as well as determining whether any of these developmental steps become impaired in disease. </p>		

Project Description:Objectives:

The transmission of electrical impulses along a nerve or muscle fiber is mediated by specialized proteins in the membrane known as ion channels or excitable sites. The electrophysiological properties of these sites or channels have been extensively studied, but the molecular characteristics of these specialized proteins have yet to be elucidated. It is the general goal of this research to elucidate the molecular nature of these proteins which are so critical in the function of nerve and muscle cells. We have focused the work on one particular such protein, frequently referred to as sodium channel, because the pharmacology of this protein is particularly well developed. This protein is known to bind several neurotoxins, which can be radioactively labeled, thus providing us with several sensitive molecular probes for this protein.

One approach in the molecular characterization of membrane proteins is a developmental one. The developmental predecessors of nerve and muscle cells, myoblasts and neuroblasts, are inexcitable cells which lack the specialized membrane proteins which mediate electrical excitability. During the process of cellular differentiation, however, these cells gradually acquire these proteins and eventually become mature, excitable nerve and muscle cells. A specific objective of this work, is to follow and study the various cellular and molecular events occurring during the process of myoblast and neuroblast differentiation which ultimately renders these cells electrophysiologically excitable. Upon reaching such an objective, we will be able to understand the biologically important molecular aspects of ion-channel proteins and, thus will be able to determine whether any of these developmental processes are impaired in disease.

Methods Employed:

- (1) Radioactive ligand binding techniques using ^3H -saxitoxin and ^{125}I -scorpion toxin.
- (2) Techniques for studying neurotoxin-stimulated ^{22}Na -uptake by neuroblastoma cells.
- (3) Standard methods for purification of membrane proteins, including solubilization of these proteins with non-ionic detergents, chromatography on ion-exchange, molecular sieving and lectin affinity columns.
- (4) Standard tissue culture techniques to grow neuroblastoma cells and chick muscle cells.
- (5) Standard electrophysiological techniques to impale cells with microelectrodes and record spontaneous and evoked action potentials.

Major Findings:

Based on toxin binding studies and on electrophysiological data, we found evidence which suggests that muscle cells synthesize an electrophysiologically inactive form of a sodium channel protein which is inserted into the plasma

membrane and, there, undergoes a post-translational modification which renders it electrophysiologically active. In order to identify the nature of this post-translational modification, we purified this protein. However, since muscle tissue contains a low density of this protein, we purified it from a tissue which is rich in the protein, namely rat brain. We have so far been able to substantially purify this ^3H -saxitoxin binding protein from rat brain and found that it has one subunit of molecular weight of 270,000 daltons and two other subunits of around 38,000 daltons. We found that rats are born with only about 10% of adult levels of this protein. They reach adult levels by around day 15. In comparing the molecular characteristics of this protein from adult versus neo-natal rat brain, we found at least one substantial difference: the protein from adult brains sticks to wheat germ agglutinin lectin column, whereas that from neo-natal tissue does not. This finding suggests that the protein from adult tissue is a glycoprotein which contains at least one carbohydrate residue not found on the protein from neo-natal material.

Scientific Significance and Relevance to Public Mental Health:

Although extremely detailed descriptions of the electrical events occurring during nerve and muscle excitation have been described in the literature, virtually nothing is known about the proteins involved in these processes. The work described in this project is aimed at elucidating, for the first time, the nature of the proteins involved in nerve and muscle excitation. The significance of this work, thus lies in the fact that it is a first attempt at understanding the biochemistry underlying the process of nerve and muscle excitation, processes that are clearly vital to the survival of man.

Proposed Course:

We are planning to continue work on the characterization of the molecular differences between adult and neo-natal ^3H -saxitoxin binding proteins. These plans include comparing the two-dimensional gel electrophoresis profile of the two forms of this protein, their behavior on sucrose density-gradient ultracentrifugation and their behavior on lectin affinity columns. In addition, we are planning to raise monoclonal antibodies directed at each of the two forms of this protein. With such antibodies, we plan to do further biochemical characterizations of this protein, further developmental studies and immunocytochemical studies.

A second series of experiments that we have planned, include determining whether this ^3H -saxitoxin binding protein undergoes any molecular changes in demyelinating diseases such as multiple sclerosis or in dystrophic muscle.

Publications:

Baumgold, J., Parent, J. B. and Spector, I.: Development of sodium channels during differentiation of chick skeletal muscle in culture: I. Binding Studies. J. Neurosci. 3: 995-1003, 1983.

Baumgold, J., Parent, J. B. and Spector, I.: Development of sodium channels during differentiation of chick skeletal muscle in culture: II. $^{22}\text{Na}^+$ -uptake and electrophysiological studies. J. Neurosci. 3: 1004-1013, 1983.

Baumgold, J., Zimmerman, I. and Bambrick, L.: Appearance of ^3H -saxitoxin binding sites in developing rat brain. Develop. Brain Res, in press, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01036-11 LNB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Regulation of Protein and Nerve Function by Modulator-Sites on Complex Carbohydrate

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Audrey L. Stone, Research Chemist, Laboratory of Neurobiology, NIMH

COOPERATING UNITS (if any)

Harvard Medical School, Massachusetts Institute of Technology,
University of Maryland, Department of Chemistry,
Laboratory of Cerebral Metabolism, NIMH

LAB/BRANCH

Laboratory of Neurobiology

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.7

PROFESSIONAL:

1.1

OTHER:

0.6

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☒ (b) Human tissues☐ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The heparin and heparan sulfate class of glycosaminoglycans (mucopolysaccharides) has an extraordinary variation in the secondary features of the disaccharide repeating units. This class of complex carbohydrates has been implicated in the modulation of numerous reactions involving enzymes, proteins and cell membranes. We have continued to characterize oligosaccharides from these glycosaminoglycans and to relate their specific chemical properties to the multiple biological functions of the macromolecule(s). The molecular basis of modulating reactions between heparin and proteins (e.g., antithrombin, tyrosine hydroxylase) is investigated by uv circular dichroism (CD) spectroscopy of the protein and its complexes with oligosaccharide modulators. Heparin has two functional domains (contained within an octadecasaccharide segment) that are able to modulate differentially the reactions between the clotting inhibitor, antithrombin (AT), and the various esterases of the clotting cascade. Using our human AT and newly-derived heparin segments of 8 to 22 saccharides, we showed that the two functional domains interact with different regions of AT. The major domain interacts with at least one 'exposed' and one 'buried' tryptophane residue of AT, while the adjacent oligosaccharide modulator appears to perturb the CD of a disulfide bond. Thus, the multifunctional action of heparin is probably related to its ability to induce conformational changes in AT. The structures of heparin and heparan sulfate oligosaccharides are investigated by low uv CD spectroscopy along with model compounds. We elucidated the saccharide sequence of the second functional domain of heparin, enabling the proposal of the sequence of the highly active, 6500 molecular weight heparin. The multifunctional modulator consists of alternating regions of higher and lower anionic density and varies in the distance between the highly sulfated segments that flank both functional domains. This important structure-function relation may have general biological significance. Conformational aspects of these domains are explored using our techniques of Induced CD spectroscopy combined with computer-calculated theoretical spectra based on the geometries of the arrays of their anionic groups. Heparan sulfate oligosaccharides from Sanfilippo patients are isolated and characterized for experiments on neuronal development.

Project Description:

Others engaged on this project are Robert D. Rosenberg, M.D., Prof. of Medicine & Prof. of Biochemistry, Harvard Medical School & MIT, David Beeler, Res. Tech., Harvard Medical School, Howard DeVoe, Assoc. Prof. of Chemistry, Univ. of Maryland, and Russell E. Martenson, Res. Chemist, LNC, NIMH.

The project concerns the structure and function of the heparin and heparan sulfate-like complex carbohydrates and how they might relate to neuronal function and development. This class of mucopolysaccharides (glycosaminoglycans) occurs intracellularly and in the cell membrane and are related by a common biosynthetic pathway that builds up their similar repeating disaccharide chains. In addition, numerous secondary biosynthetic changes in the structural elements lead to a recently revealed, extraordinary variability in oligosaccharide sequences that is expressed quantitatively and qualitatively and within a given molecule as well as among the different heparin-like glycosaminoglycans (GAG). The variations have yet to be classified and related to the biological functions of this important class of macromolecules. These GAG are known to modulate numerous reactions involving enzymes and proteins (e.g., tyrosine and tryptophan hydroxylases and antithrombin). Heparan sulfates are also implicated in cell-cell interactions, cell migration and fixation of enzymes to the cell membrane. They are present in cell membranes, microvessels and mast cells of the CNS, and in certain basement membranes. It is speculated that the multifunctional reactivity of this class of complex carbohydrates derives from the chemical properties of various unique oligosaccharides (OligoS) that reside within the macromolecule. By virtue of their polyanionic nature, however, these mucopolysaccharides (MPS) are also involved in binding and exchange of simple and complex cations in interstitial elements.

Objectives:Subproject 1. Modulation of the Anticoagulant Reactions of Antithrombin by Heparin.

To determine the molecular basis of the modulation of the reactions between antithrombin (AT) and the serine esterases of the clotting cascade; to form complexes between purified, heparin-derived oligo- and mucopolysaccharides and human AT and determine the conformational aspects of the unmodified and modified protein; to elucidate the molecular basis of activation of CNS tyrosine hydroxylase by heparin; to develop models of protein-polysaccharide interactions that may occur during developmental processes such as neuronal cell migration.

Subproject 2. Structure-Function Relations of Modulator Sites in Heparin-type Complex Carbohydrates. To determine and characterize the saccharide sequences of various heparin-derived OligoS that are able to modulate differentially the anticoagulant potency of AT; to reveal polysaccharide features (conformational and structural) of other unusual amino-sugar containing GAGs.

Subproject 3. Role of Heparan Sulfate in Neuronal Development and Function. To initiate studies of heparan sulfates as modulators of enzymatic and/or developmental processes in the CNS; in particular to characterize oligo- and polysaccharide heparan sulfate fractions from patients with Sanfilippo mucopolysaccharidoses and to identify their possible role in development, especially as it might relate to the mental retardation associated with these disorders.

Methods Employed:

Standard chromatographic and analytical techniques were used to isolate proteins, heparin fractions of 22 Kd and 6500d and other complex carbohydrates. OligoS were generated by controlled nitrous acid cleavage of heparin. Assays of biological activity of the coagulation enzymes and heparins were spectrophotometric (developed by Prof. Rosenberg), while that for tyrosine hydroxylase was a radioassay developed at NIH using sub-optimal cofactor levels. The conformational aspects of the proteins were measured by near ultraviolet (uv) circular dichroism (CD) spectroscopy using a modified Cary model 60, the Cary model 61 and the Jasco 500J spectropolarimeters, under newly specified conditions. The Perkin-Elmer spectrofluorimeter and Cary 118C spectrophotometer were also employed. The structures of the sequential disaccharide repeating units of the biologically active OligoS were determined by low uv CD spectroscopy in combination with optical models of the various repeat units.

The number of anionic charges of the complex carbohydrates and their distribution were determined by titration using the metachromatic cationic ligands; conformational aspects of the bound ligand and its binding site were examined by the techniques of extrinsic circular dichroism spectroscopy we previously developed. A computer program was newly established with Prof. H. DeVoe to predict the extrinsic CD spectra and metachromatic absorption spectra of the ligands bound to various groups of anionic sites on heparin and heparin-like MPS. The prediction was based upon changes in absorption of ligands due to coulombic interactions between the light-absorbing monomers (coupled oscillators).

Major Findings:

Subproject 1: Many results were given in the previous annual report. The reactions of the clotting inhibitor, AT, with the serine esterases of the clotting cascade were known to be markedly accelerated by the binding of heparin to AT. Heparin had at least two functional domains that were contained within an octadecasaccharide segment and that were able to modulate differentially the anticoagulant potency of AT. The first domain contains a unique hexasaccharide that is essential for binding of heparin to AT. This OligoS is necessary and sufficient for accelerating the reaction between AT and factor X_a but is without effect on factor XI_a , IX_a and thrombin-antithrombin interactions. Binding of a second domain, present in OligoS larger than the hexadecasaccharide, is required for the acceleration of reactions between AT and factors XI_a , IX_a , and thrombin (previous findings by Prof. Rosenberg). Our findings reveal that heparin induces two types of chiro optical effects in AT that correlate with the binding of the two functional domains. OligoS smaller than the hexadecasaccharide (8-to-14 saccharides) induce the same alteration in AT. The reaction causes the perturbation of at least one of the two "buried" and one of the two "exposed" tryptophan residues in the protein. OligoS modulators larger than the hexadecasaccharide (the octadecasaccharide, the 22-saccharide (6500d) fraction and the 22,000d fraction) induce additional alterations in the CD spectrum of AT that is consistent with perturbations of a disulfide bridge. Thus, our CD data show that the two functional domains of heparin probably interact with separate areas of AT and appear to induce different degrees of alteration in the structure of the protease inhibitor.

Heparin was also known to accelerate the reactions of tryptophane hydroxylase and of tyrosine hydroxylase (TH) via a marked decrease in the K_m of their cofactor, tetrahydrobiopterin. We discovered that cations containing lysyltyrosine groups markedly inhibited TH, that heparin reversed this inhibition and that a naturally occurring 'low capacity' form (having a low K_i for lysyltyrosine and a high K_m for substrate) could be isolated from striated tissue (detailed in a previous annual report). We proposed that the *in vitro* regulation of TH occurs because of reversible conformational constraints imposed by electrostatic interactions within the enzyme protomer. We now find that there is a degree of specificity in the polycation inhibition and that the geometry of the negative charges among a number of polyanions appears to be critically important in the activation reaction. Most striking is the biphasic effect of polyglutamyltyrosine. This polypeptide exhibited marked stimulation at concentrations up to 0.1 ugms/ul followed by a heparin-insensitive inhibition (down to 11 per cent of control at 1 ugms/ul). Thus, potential for either activation or inhibition of TH can reside in the same effector molecule and the effect would depend upon the enzymic capacity of the TH.

Subproject 2: Low uv CD of a series of OligoS fragments (octa-, deca-, dodeca-, tetradeca-, and octadecasaccharides) from heparin reveals the disaccharide sequences residing in the second binding domain of the MPS. These appear to be a fully-sulfated N-sulfaminoglucosaminyl-iduronic acid, a non-sulfated iduronyl-N-acetylglucosamine, a glucuronyl-glucosamine, and two highly sulfated disaccharides, respectively. Together with the structure of the octasaccharide, these components yield the sequence of the octadecasaccharide which shows that both domains have two non-sulfated uronic acids and N-acetylglucosamine flanked by fully-sulfated disaccharides. This sequence analysis along with other analytical data permits us to propose a tentative structure of the fully potent 6500 molecular weight fraction of heparin. Two major structure-function inferences can be drawn. The first points out that alternating regions of relatively low and high anionic density may be the hallmark of binding domains in these complex carbohydrate chains. The second inference suggests that difference in distance between specific sulfate groups that flank the region of lower anionic density is signal for each binding sequence in the multifunctional modulators.

Extrinsic CD in metachromatic ligands bound to heparin-like MPS and OligoS were known to be dependent upon the geometry of the negative charges of the polyanions in solution. There are numerous possible groupings of the ligands (bound to the neighboring anionic sites) that might give rise to the observed induced CD. We calculated spacial coordinates for the four chemically distinct anionic groups of the heparin disaccharide in accordance with X-ray crystallography data which shows the chain to have a two-fold axial repeat of disaccharide units. With Dr. DeVoe, we treat these positions as point dipoles in a theoretical model of ligand-ligand interactions. We demonstrate that predicted extrinsic CD spectra for the various arrays of negative sites can be systematically calculated to determine inappropriate or appropriate geometries of the neighboring ligands that give rise to the asymmetric absorption exhibited by methylene blue (MB): heparin complexes. Thus far we show that the ligand, (MB), is most probably bound at one of its amino groups, not at the internal nitrogen of the aromatic ring system, that more than two of the types of anionic sites are involved in the asymmetric array, and that a C₆ O-sulfate and a sulfamino group are critical sites.

The laboratory facilities were moved to a new module centered within the LNB and the recently acquired Cary 60 spectropolarimeter which is partially converted to solid state electronics was installed and corrected to optical specifications. These were the more time consuming because of lack of support staff until after the move. Progress in Subproject 3 was also slowed down this year because of lack of staffing.

Subproject 3: OligoS of 4-20 saccharides in size were isolated from the urinary heparan sulfates of Sanfilippo A and B patients (described in the previous annual report). Their characterization and plans to study their role in neuronal development are in progress.

Scientific Significance and Relevance to Public Mental Health:

Over a number of years our investigations have elucidated the structural and physicochemical properties of the various macromolecular MPS in relation to their diverse biological functions. Our efforts have provided information about the special attributes of each of the four major classes of GAG and a number of novel techniques for the study of the relationship between their structure and function. Recently, we have been advancing techniques particularly for investigation of the molecular basis of the biological functions of the heparin-class of complex carbohydrates. The findings of Dr. Rosenberg were seminal to our new understanding of the structure-function relationships of this class of MPS. However, the reactions of heparin in promoting anticoagulation must be considered prototypical of those that can be ascribed to these complex carbohydrate in other biological systems. These remarkable macromolecules have, at the same time, the ability to undergo three types of reactions. The first involves specific reactions between the polysaccharide and protein that alters the reactivity of the protein. The second involves promoting protein-protein reactions by virtue of having specific binding sites for both proteins, which brings them in close approximation. The third type of reactivity is that of selective self-aggregation among various OligoS segments. Our hypothesis regarding the role of these OligoS modulators in mental health relates these special abilities which we are studying to the complex process of cell migration in the developing CNS. We believe that the heparin-class of macromolecules are involved in defining the routes between the neuron and target cell.

We first show that, indeed, the two functional domains of the heparin octa-decasaccharide segment react with different portions of AT and probably selectively alter the integrity of the protease inhibitor. Further analysis and a manuscript entitled "Absorption and Circular Dichroism Spectroscopy of Anticoagulant Heparin Complexes with Aromatic Polypeptides and with Human Antithrombin" by A. L. Stone, R. Osborne, D. Beeler and R. D. Rosenberg are in progress.

We then show that the striking variability in saccharide sequences exhibited by this class of complex carbohydrates appears to have an orderliness. Short segments of relatively low anionic density (and having an N acetylglucosamine moiety that is capable of reacting with the indole nitrogen of a tryptophane residue in a protein) are flanked by a different number of highly sulfated disaccharide repeating units. Thus, we propose that these "variable regions" are not only responsible for the multifunctional actions of a given macromolecule but may also be an expression of predetermined specificity (information content).

Our investigations are at the probing edge in other aspects of this new area of research. We characterize heparan sulfate fragments with the view toward exploring the possible role of these OligoS and macromolecules in the development of the CNS. Our computer analysis of the theoretical induced CD spectra of was developed with Dr. DeVoe not only to determine which array is responsible for the overall pattern seen with heparin but also to develop a series of probes that are selective for the presence and frequency of particular OligoS sequences that might occur in a number of macromolecules. These results will be presented at the Annual Meeting of the Society for Complex Carbohydrates, October, 1983 in a paper entitled "Theoretical Predictions of the Extrinsic Cotton Effects of Methylene Blue Complexes with Oligosaccharide Segments of Heparin and Heparan Sulfate" by A. L. Stone, E. L. Winter and H. J. DeVoe.

With Dr. Martenson studies of the conformations of myelin basic protein and several of its peptide fragments are in progress. If successful the results will enable him to better understand the relationship between the secondary structure of these peptide regions and the compaction of myelin basic protein in the myelin sheath of the CNS.

Proposed Course:

Subproject 1. The study of the molecular basis of heparin modulation of AT will be continued with the view towards completion of this aspect of the project. CD analysis of the conformational forms of oligopeptides from myelin basic protein in collaboration with Dr. R. Martenson will be continued. Further efforts to elucidate the regulation of tyrosine hydroxylase by complex carbohydrates awaits the availability of a highly purified enzyme.

Subproject 2. Sequence analysis of OligoS modulators of heparin and heparan sulfate, analysis of charge distributions in these OligoS, and the theoretical predictions of extrinsic CD will be continued.

Subproject 3. The OligoS of Sanfilippo mucopolysaccharidoses from 4 to 20 saccharides will be further characterized by CD spectroscopy and other structural probes; with a visiting fellow, investigation of the possible role of these and other OligoS in neuronal development will be undertaken using several neurobiological systems.

Publications:

Stone, A. L., Beeler, D., Oosta, G., and Rosenberg, R. D.: Circular dichroism spectroscopy of heparin-antithrombin interactions. Proc. Natl. Acad. Sci., USA 79: 7190-7194, 1982.

Stone, A. L., Beeler, D., and Rosenberg, R. D.: Physical properties of the oligo- and polysaccharide heparin fractions that bind to and activate human antithrombin. I. Far ultraviolet circular dichroism spectroscopy and analysis of uronic acid sequences. (Submitted to Biopolymers)

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01031-15 LNC

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Conversion of Phenylalanine to Tyrosine

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Seymour Kaufman, Chief, Laboratory of Neurochemistry, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

5.3

PROFESSIONAL:

4.3

OTHER:

1.0

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard un-reduced type. Do not exceed the space provided.)

Tetrahydrobiopterin (BH_4) has been found to inhibit the activation of phenylalanine hydroxylase that is brought about by limited proteolysis and by phosphorylation; phenylalanine overcomes this inhibition. Phenylalanine hydroxylase is in an activated state in livers from diabetic rats. Administration of insulin to these rats de-activates the enzyme. Rat liver phenylalanine hydroxylase has been shown to be composed of identical subunits.

OTHER PROFESSIONAL PERSONNEL:

Masayoshi Iwaki	Visiting Fellow	LNC NIMH
Brucé Gomes	Staff Fellow	LNC NIMH
Harvey Wilgus	Senior Staff Fellow	LNC NIMH
Desirazu Narasimha Rao	Visiting Fellow	LNC NIMH
Robert Phillips	Staff Fellow	LNC NIMH
Michael Parniak	Visiting Associate	LNC NIMH
Frank Gold	Chemist	LNC NIMH
Lorraine Moberly	Chemist	LNC NIMH

Project Description:

The objective of this research project is the detailed description of the enzyme system that catalyzes the conversion of phenylalanine to tyrosine. The specific goal is the analysis of the structure, mechanism of action, and modes of physiological regulation of the essential components in the hydroxylation system. These components include phenylalanine hydroxylase, dihydropteridine reductase and tetrahydrobiopterin (BH_4).

One of the reasons why the regulation of this system is of special interest to neurochemists is that it can serve as a paradigm for the dynamic interaction between metabolic events in peripheral organs and the brain. When this interaction goes awry, as it does in classical phenylketonuria, it can lead to severe mental retardation.

Major Findings:

There have been reports from several different laboratories that hepatic phenylalanine hydroxylase is composed of two different subunits, Mr of each one being approximately 50,000-55,000. We have now shown by analysis of peptides obtained by cleavage of the pure hydroxylase with cyanogen bromide that there is only a single polypeptide. These results suggest that the previous evidence for subunit heterogeneity was due to artifacts. Further structural studies on the enzyme have shown that the N-terminal amino acid residue is blocked by a still unidentified blocking group.

We have found that tetrahydrobiopterin (BH_4) markedly inhibits the activation of rat liver phenylalanine hydroxylase by chymotrypsin, whereas L-phenylalanine slightly stimulates. Phenylalanine is able, however, to completely overcome the inhibitory effects of BH_4 . Very similar results were obtained when the effects of BH_4 and phenylalanine on the ability of cAMP-dependent protein kinase to activate and phosphorylate phenylalanine hydroxylase were studied: BH_4 inhibits and phenylalanine is able to overcome the inhibition.

Since it is known that insulin and glucagon often work in opposition to each other, we anticipated that in diabetic rats, phenylalanine hydroxylase should be in an activated state, because in such animals glucagon, which we have previously shown can activate the hydroxylase, would be working unopposed by insulin. We have found that the BH_4 -dependent phenylalanine hydroxylase activity in diabetic rats is about two-fold higher than it is in control animals and that insulin administration to the diabetic rats lowers the activity toward normal levels.

Significance to Biomedical Research and Proposed Course of Project:

The effects of ligands such as BH_4 and phenylalanine on the ability of phenylalanine hydroxylase to be activated by limited proteolysis and by cAMP-dependent protein kinase indicates that the state of activation of the enzyme in vivo is determined by the balance between inhibition by BH_4 and relief of this inhibition by phenylalanine. These results, especially those with cAMP-dependent protein kinase, suggest that in the basal state the hydroxylase is kept in a relatively low activity form by BH_4 . In response to an elevation in tissue levels of phenylalanine, the hydroxylase is activated by phosphorylation. In this process, phenylalanine plays two key roles: a) it stimulates the release of glucagon from the pancreas; b) it overcomes the inhibition by BH_4 of the ability of the kinase to activate the hydroxylase. This type of control mechanism would prevent the depletion of the organism's relatively small pool of free phenylalanine, a depletion which would bring protein synthesis to a halt.

The finding that insulin can de-activate phenylalanine hydroxylase demonstrates for the first time that this enzyme is under multiple hormonal control, i.e., glucagon and insulin. Although we have shown that the glucagon effect is probably mediated by this hormone's ability to stimulate hepatic adenylate cyclase, and, hence, to elevate hepatic levels of cAMP, the mechanism of this inhibitory effect of insulin is not known. We intend to study the mechanism of this effect in isolated rat hepatocytes.

It would be important to extend the finding that phenylalanine hydroxylase is in a more activated state in diabetic rats to whole animals to see if that activation is expressed. We plan to do this using the whole animal perfusion technique for studying phenylalanine metabolism that we have previously developed. If the activation is expressed in vivo, it would be of interest to determine whether this altered activity has any other metabolic consequences for the organism.

Publications:

1. Hasegawa, H., Kaufman, S.: Spontaneous activation of phenylalanine hydroxylase in rat liver extracts. J. Biol. Chem., 257, 3084-3089, 1982.
2. Kaufman, S., Shaw-Goldstein, L.: Oxidation-reduction reactions of 2,4,5-triamino-6-hydroxypyrimidine and its cofactor activity in the phenylalanine hydroxylase system. Proceedings of the Third International Symposium on Oxidases and Related Redox Systems (ISOX); T. E. King, H. S. Mason, and M. Morrison (eds.) Pergamon Press, New York, 1982, pp. 543-562.
3. Kaufman, S., Mason, K.: Novel amino acid substrates and activators for rat liver phenylalanine hydroxylase in oxygenosis and oxygen metabolism. M. Nozaki, S. Yamamoto, Y. Ishimura, M. J. Coon, L. Ernster, R. W. Estabrook (eds.) Academic Press, New York, 1982, 305-318.
4. Kaufman, S., Mason, K.: Specificity of amino acids as activators and substrates for phenylalanine hydroxylase. J. Biol. Chem., 257, 14667-14678, 1982.
5. Phillips, R. S., Iwaki, M., Kaufman, S.: Ligand effects on the limited proteolysis of phenylalanine hydroxylase: evidence for multiple conformational states. Biochem. Biophys. Res. commun., 110, 919-925, 1983.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01034-15 LNC

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The Biochemical Basis of Skeletal Muscle Hypertrophy

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Seymour Kaufman, Chief, Laboratory of Neurochemistry, NIMH

COOPERATING UNITS (if any)

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.6

PROFESSIONAL:

1.2

OTHER:

0.4

CHECK APPROPRIATE BOX(ES)

- ☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unexpanded type. Do not exceed the space provided.)

The object of this work is the elucidation of the biochemical mechanism of compensatory skeletal muscle hypertrophy. These studies will give greater insight into the physiological regulation of normal and pathologic skeletal muscle metabolism and into the role of the nervous system in this regulation. They may also prove to be generalizable to the study of adaptation of other organs and tissues to increased physiological demand (e.g., the nervous system).

Present topics of investigation include:

- 1) Chemical characterization of growth-factor-like activity from extracts of hypertrophied rat hindlimb muscle.
- 2) Chemical characterization of a similar activity from horse serum.
- 3) Determination of the relationship between such substances and the process of stretch-induced hypertrophy as measured by amino acid uptake and incorporation into protein, cellular content of contractile protein and cation transport.

OTHER PROFESSIONAL PERSONNEL:

Michael G. Bissell, M.D., Ph.D.
Rosanne Bailey

Senior Staff Fellow
Physical Science Tech.

LNC NIMH
LNC NIMH

Project Description:

Passive mechanical stretching of skeletal muscle leads to hypertrophy of the tissue in response. Using a technique developed in this laboratory for stretching monolayers of chick embryo myotubes in vitro, we have shown that skeletal myotubes respond to passive stretch by increased amino acid uptake (as measured by uptake of amino-isobutyric acid, AIB), increased incorporation of amino acids into, and accumulation of total cellular protein. These increases are inhibited by ouabain after a 30-minute lag period and by tetrodotoxin. Stretch is also associated with an early increase in the V_{max} of the membrane Na/K-dependent ATPase, as measured by Rubidium-86 uptake. These stretch effects take place in serum-free medium and are mimicked by the addition of serum to unstretched cultures.

More recently, we have carried out additional studies on a whole animal model of skeletal muscle hypertrophy. This in vivo tenotomy model involves the surgical section of the Achilles tendon of the gastrocnemius muscle of one limb while a "sham" operation is carried out on the other limb. Following the operation, the weight-bearing load is redistributed from the gastrocnemius to the two smaller synergist muscles, the soleus and plantaris, which rapidly hypertrophy. Using this system, we have seen significant increases in wet weight of the soleus and plantaris, as early as four to six hours post-tenotomy.

Major Findings:

We have made aqueous extracts of hypertrophied soleus and plantaris muscles from rats 48 hours after cutting the tendon. These extracts, like serum, cause dose dependent increases in AIB uptake and amino acid incorporation when added to unstretched myotube cultures. Similar extracts from the corresponding nonhypertrophied muscles on the unoperated contralateral hindlimbs of the same animals show a significantly smaller effect when added to the cultures. The substance (or substances) responsible for this effect, like the similarly active serum component, is heat labile and retained by a dialysis bag. The active factor(s) from rat hindlimbs is degraded by treatment with -chymotrypsin. Its effect on AIB uptake is not additive with 10% stretch. Like the effects of stretch and serum on AIB uptake, its effect is sensitive to cycloheximide, actinomycin D and ouabain, but unlike stretch, it is effective within 5 minutes of application. These substances may represent intracellular proteins which mediate the stretch response, but their precise relationship, if any, to the stretch response has yet to be determined.

Significance to Biomedical Research Proposed Course of Project:

The finding that hypertrophied muscle appears to contain substances that can stimulate many, and perhaps all, of the biochemical effects induced by stretching muscles, promises to help in elucidating the mechanism of the stretch response. The responsible substance(s) elaborated by hypertrophied muscles may have growth stimulating effects on other tissues. We plan to explore this possibility. We also intend to try to characterize the substance(s) responsible for these effects.

Publications:

1. Vandenburg, H. H., and Kaufman, S.: Coupling of voltage-sensitive sodium channel activity to stretch-induced amino acid transport in skeletal muscle in vitro. J. Biol. Chem. 257, 13448-13454, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01035-15 LNC
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Process of Lysogeny		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Howard Nash, M.D., Ph.D., Laboratory of Neurochemistry, NIMH		
COOPERATING UNITS (if any) Laboratory of Molecular Genetics, NICHD; Laboratory of Molecular Biology, NCI; Laboratory of Molecular Biology, NIAMDD; Roche Institute of Molecular Biology; Department of Chemistry, Carnegie-Mellon University		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.0	PROFESSIONAL: 3.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The way in which segments of <u>DNA</u> find each other to initiate site-specific <u>genetic recombination</u> has been explored. Two pathways have been defined; random collision and one-dimensional sliding along DNA. The choice between these two pathways reflects differences in the nucleosome structure of the recombining sites caused by <u>recombination proteins</u>. The interaction of one recombination protein with sequences not involved in recombination has been examined. A <u>consensus sequence</u> for the binding of this protein has been deduced. <u>Methylation protection</u> experiments indicate that binding of this protein is unusual in that the primary contacts involve residues of the protein with the <u>minor groove</u> of the double helix. </p>		

OTHER PROFESSIONAL PERSONNEL:

Nancy Craig	Staff Fellow	LNC NIMH
Brenda Lange-Gustafson	Staff Fellow	LNC NIMH
Paul Kitts	Visiting Fellow	LNC NIMH
Carol Robertson	Biologist	LNC NIMH

Project Description:

Lysogeny refers to the stable association between the bacterial virus lambda and its host E. coli. This association requires both the repression of viral genes and the integration of the viral genome into the host chromosome. Integration is achieved by a reciprocal recombination between specific sequences, called attachment sites, located on the phage and host chromosomes. Because of its simplicity and high efficiency, integrative recombination of lambda is a valuable model system for studying the mechanism of site specific genetic recombination, a phenomenon with wide biological distribution. Our laboratory was the first to demonstrate that this reaction could take place in cell-free extracts and we have subsequently identified and purified the components of this recombining system. This achievement has permitted us to explore in more detail the way in which DNA strands are broken, aligned with new partners, and rejoined.

Previous studies on the interaction of recombination proteins with DNA have demonstrated that attachment sites are festooned with protein: at the viral attachment site (attP) at least 300,000 daltons of protein are clustered in a region of 240 base pairs of DNA. Several lines of evidence indicate that these proteins do not simply form a linear array on attachment site DNA but instead form a complex nucleoprotein structure like that found in the nucleosomes of chromatin. We have now discovered that the details of this higher order structure have profound consequences for the pathway of recombination. The basic experiments that address this point involve recombination between the sites that flank the integrated virus. Recombination between these prophage sites normally serves to excise the integrated viral DNA from the E. coli chromosome during the reversal of lysogeny. Using the purified proteins that were identified as components of the integration pathway, excisive recombination between prophage sites that are oriented on a single circle of DNA in a head to tail fashion is readily permitted. However, these prophage sites cannot be recombined if they are oriented on a single circle of DNA in a head to head fashion nor do they recombine when they are on separate circles. Thus, the recombining prophage sites must sense their relative orientation even though they are separated by several thousand base pairs of DNA. We conclude that the recombination machinery must slide DNA past one site until the second site is found. We propose that such sliding is a consequence of the nucleosome-like structure when a mixture of strong and weak interactions between protein and DNA fixes one part of the attachment site to the recombination apparatus but permits another part of the DNA to wander while held in loose contact with the protein. The observations with excisive recombination are in complete contrast to those observed using the same purified proteins for integrative recombination. In this case, recombination proficiency is insensitive to relative orientation. We take this to mean that random collision, not sliding, is the major pathway for the successful apposition of the viral and host attachment sites. This disparity between integrative and excisive recombination is confirmed by a comparison of the circular products of recombination

between directly repeated sites. For integrative recombination the product circles are catenated to one another, reflecting the random trapping of loops of DNA during the juxtaposition of the two attachment sites; in excisive recombination, the circular products are separate, reflecting the orderly migration of one site to the other. We believe that because the prophage sites contain different combinations of binding sequences for recombination proteins than do the viral and host sites, recombination proteins organize the two different kinds of attachment sites into two different kinds of nucleosome-like structures. As a result, the mechanism by which sites find one another is completely altered.

Our earlier studies demonstrated that the E. coli protein required for in vitro site-specific recombination, integration host factor or IHF, is a DNA binding protein that specifically interacts with sequences within attachment sites. In an attempt to shed light on the role of this host protein in uninfected cells, we have extended our studies to inquire about the interaction of IHF with non-attachment site DNA. Two additional specific binding loci have been found. Both are located in regions of DNA concerned not with recombination but with the control of gene expression. This finding fits well with the in vivo observation that mutants of E. coli that have an altered IHF show deranged expression of several genetic loci. Comparison of the sequences bound by IHF at three regions of attP and the two additional sites indicates that the sequence AANNNTTGAT directs the binding of IHF to DNA (N indicates any of the four common bases). In several cases we have shown that regions of DNA that contain sequences that differ from this consensus binding sequence by two base pairs do not bind IHF. However, some of the non-binding regions are from genetic loci that depend on IHF for normal regulation in vivo. This suggests that binding to DNA may not be the only way that IHF affects gene expression. Another indication that IHF may be an unusual regulatory protein has come from our analysis of the intimate contacts between DNA and IHF. In those regions where IHF specifically binds to DNA, we have shown that IHF can protect certain nucleotides from chemical attack by the alkylating agent dimethyl sulfate. However, in contrast to the usual protection of guanine residues that has been observed in many studies of the interaction between DNA and repressors, activators, and polymerases, IHF preferentially protects adenines. This means that the primary contacts between IHF and DNA are in the minor groove of the double helix and not in the major groove, the site of contact between DNA and most previously studied regulatory proteins.

Significance to Biomedical Research and Proposed Course of Project:

Site-specific recombination is fundamental to several important biological processes. It is one of the few ways to introduce flexibility into hereditary material and, as such, is used to select certain regions of the genome for: (a) reconstruction to produce new genes, (b) specific replication to amplify the copy number of certain genes, and (c) relocation of genes to alter their pattern of expression. Our studies bring to light the way in which site-specific recombination is achieved in at least one biological setting.

We will continue to inquire about the way recombination proteins interact with DNA. Our new emphasis will be on learning about the higher order structure imposed by these proteins on the recombining DNA. The first step will be to determine the way

these proteins alter the twist and writhe of DNA helix. In addition, we will begin to explore the mechanism by which IHF regulates gene expression. Collaborative studies with several laboratories that are dedicated to gene regulation are under way. Finally, we will begin to use new chemical tools to modify the DNA attachment sites in order to probe for the chemical features that are required for recombinant function.

Publications:

1. Nash, H. A.: Purification and properties of the bacteriophage lambda int protein. In: Recombinant DNA, R. Wu, L. Grossman, and K. Moldave, (eds.) Methods of Enzymology, 100, 210-216 (1983).
2. Pollock, T. J. and Nash, H. A.: Studies on the role of DNA supercoiling in lambda integrative recombination. In: Genome Rearrangements (D. A. Hopwood, C. A. Cullis, A. W. B. Johnston, H. W. W. Woolhouse, K. F. Chater (eds.) Proceedings of the Fifth John Innes Symposium. John Innes Charity, Norwich, Croon Helm (London), p. 41-58, 1983.
3. Pollock, T. J. and Nash, H. A.: Knotting of DNA caused by a genetic rearrangement: Evidence for a nucleosome-like structure in site-specific recombination of bacteriophage lambda. J. Mol. Biol., (in press).
4. Nash, H. A. and Pollock, T. J.: Site-specific recombination of bacteriophage lambda: The change in topological linking number associated with exchange of DNA strands. J. Mol. Biol., (in press).
5. Craig, N. L. and Nash, H. A.: The mechanism of phage lambda site-specific recombination: Collision versus sliding in att site juxtaposition. In: UCLA Symposium on Cellular and Molecular Biology. Fox, C. F. and Cozzarelli, N. (eds.) 1983., (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01037-15 LNC
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Role of the Cell Membrane in Cellular Organization, A Molecular Study.		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) David M. Neville, Jr., Chief, Section of Biophysical Chemistry, LNC, NIMH		
COOPERATING UNITS (if any) Minnesota Bone Marrow Transplantation Group		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION Biophysical Chemistry		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 5.0	PROFESSIONAL: 4.0	OTHER: 1.0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The general aim of this project is to determine the chemical interactions which occur at the <u>surfaces of cells</u> which affect cellular <u>differentiation</u> and organization. Specifically we have studied one type of interaction, <u>plasma membrane receptor mediated entry of proteins into the cell cytosol</u>. These studies have been done by developing techniques to construct artificial <u>protein hybrids</u> containing the active fragment of a <u>toxin</u> and another receptor specific binding protein. Such artificial protein hybrids have value as a new class of <u>pharmacologic reagents</u>. <u>Monoclonal antibody ricin</u> conjugates directed against human <u>T cells</u> effectively eliminate these cells from donor bone marrow permitting <u>bone marrow transplants</u> free from <u>graft versus host disease</u>. This will provide a new treatment for <u>leukemia</u>, <u>aplastic anemia</u> and <u>autoimmune diseases</u> such as <u>multiple sclerosis</u>, <u>Guillain Barre syndrome</u>, <u>systemic lupus erythematosus</u> and perhaps other diseases of the immune system such as <u>acquired immunodeficiency syndrome</u>. In addition, these reagents are useful for <u>enzyme replacement therapy</u> and in <u>organ transplantation</u>.</p>		

OTHER PROFESSIONAL PERSONNEL:

Richard Youle	Research Biochemist	LNC NIMH
Jon Marsh	Staff Fellow	LNC NIMH
Thomas Hudson	Staff Fellow	LNC NIMH

Project Description:

The general aim of this project is to determine the chemical interactions which occur at the surfaces of cells which affect cellular differentiation and organization. The major specific aim of the program is to synthesize a new class of pharmacologic reagents which permit the modulation of specific cell types in a manner unobtainable with conventional reagents. This is being accomplished by the synthesis of artificial protein hybrids which utilize the process of receptor-mediated protein transport.

A wide variety of proteins are capable of entering cells by receptor-mediated transport processes. Having gained entry these proteins are directed to specific cellular compartments where they exert either a physiological or pathological function.

In general it appears that only a discrete portion of these proteins contain the receptor binding activity which is involved in the entry process while another portion of the protein performs the intracellular function. Therefore, it is possible to split and reassemble two such proteins with a new combination of receptor entry specificity and intracellular function. Such proteins we call artificial hybrid proteins, and previous reports from this laboratory have suggested that such hybrids should have utility both as probes of entry processes and as a new class of pharmacologic reagents with tailor made receptor and therefore cell type specificity.

Major Findings:

A mixture of anti-T cell monoclonal antibody-ricin conjugates developed in this laboratory has been used to treat human donor marrow during bone marrow transplantation in order to eliminate graft versus host disease. Early results are highly encouraging. No untoward effects of these reagents have been noted. Enlarged clinical trials are in progress.

An antigen-ricin conjugate, in this case tetanus toxoid-ricin, has been used to block a specific antibody response, anti-tetanus-toxoid in an in vitro model test system. However, cells mounting an antibody response against an unrelated test antigen were unaffected demonstrating the immunologic specificity of these reagents.

Significance to Biomedical Research:

Bone marrow transplantation is the accepted therapy today for aplastic anemia and a variety of leukemias. Autoimmune diseases of fatal outcome or high morbidity such as multiple sclerosis, Guillain Barré, systemic lupus erythematosus and other diseases of the immune system such as AIDS are all potentially curable by bone marrow transplantation. Certain enzyme deficiency states can also be cured by marrow transplantation. At present, the occurrence of graft versus host disease (GVHD) limits the applicability of

this procedure to those patients having an identical twin or an HLA matched sibling, although in the latter situation morbidity and mortality from GVHD is considerable.

It now appears that by using methods which T cell deplete donor marrow such as our reagent, methotrexate treatment need not to be given to the recipient to prevent GVHD. This results in a very mild post transplant course. This situation, if replicated by future studies will increase the applicability of bone marrow transplantation to the above listed diseases.

Proposed Course of Project:

Clinical studies using anti-T cell ricin conjugates to prevent graft versus host disease will continue and will be designed to determine (1) optimal dose, and (2) whether GVHD can be eliminated when transplants are performed across HLA barriers. If this is successful, these reagents will be used clinically to treat fatal and highly morbid autoimmune diseases. They will also be used in organ transplantation situations where the organ is often rejected (heart, lung, adult liver) by performing simultaneous bone marrow transplantation and organ transplantation within the same donor recipient pair.

Basic work is continuing to develop similar reagents for in vivo use. This will permit eradication of unwanted cell types. Modulation of the immune system as a treatment of autoimmune diseases may be possible without the use of high radiation doses.

Publications:

1. Volkman, D.J., Ahmad, A., Fauci, A.S., and Neville, D.M., Jr.: Selective abrogation of antigen-specific human B cell responses by antigen-ricin conjugates. J. Exp. Med. 156, 634-639, 1982.
2. Vallera, D.A., Youle, R.J., Neville, D.M., Jr., Soderling, C.B. and Kersey, J.H.: Bone Marrow Transplantation Across Major Histocompatibility Barriers in Mice. VI. Anti-T Cell Monoclonal Antibody-Toxin Conjugates as Reagents for Experimental GVHD Prophylaxis are not Selectively Reactive with Marine Stem Cells. Transplantation, in press.
3. Neville, D.M., Jr., Youle, R.J., Kersey, J.H., and Vallera, D.A.: Monoclonal antibody-Ricin Conjugates for the Treatment of Graft versus Host Disease. Present and Future Prospects. In: Langman R. and Delbecco, R. (eds.). The Armand Hammer Cancer Symposium, Monoclonal Antibodies and Cancer. Academic Press. 1983. (In press).

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01038-15 LNC

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Phenylketonuria and Other Disease Caused by Defects in Biopterin-Dependent Enzymes

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Seymour Kaufman, Chief, Laboratory of Neurochemistry, NIMH

COOPERATING UNITS (if any)

Section on Human Biochemical Genetics, NICHD

LAB/BRANCH

Laboratory of Neurochemistry

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:

1.0

PROFESSIONAL:

0.3

OTHER:

0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies of several children with hyperphenylalaninemia due to defects in the de novo synthesis of tetrahydrobiopterin (BH_4) have shown that the oral administration of either BH_4 or a synthetic analogue, 6-methyltetrahydropterin, leads to marked improvement of the neurological symptoms in some, but not all, of these patients. There is a correlation between the ability of the administered pterin to elevate CSF levels of the metabolites of dopamine and serotonin and its ability to improve the neurological status of the patients.

OTHER PROFESSIONAL PERSONNEL:

Lynne Binari

Biologist

LNC NIMH

Project Description:

In 1975, cases of hyperphenylalaninemia were reported in which neurological disorders persist despite dietary control of phenylalanine blood levels. Subsequently, variant forms of phenylketonuria (PKU) or hyperphenylalaninemia were described by our laboratory in which the defect in the phenylalanine hydroxylase system is not in phenylalanine hydroxylase, itself, as it is in classic PKU, but rather in dihydropteridine reductase or in an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH_4). Dihydropteridine reductase functions to maintain BH_4 in its functional tetrahydro form while BH_4 is an essential coenzyme. Both of these variants are therefore characterized by a marked deficiency of BH_4 . Since, as previous work in this laboratory had shown, this pterin is an essential coenzyme not only for phenylalanine hydroxylase, but also for tyrosine and tryptophan hydroxylases, patients lacking BH_4 suffer from defects in the synthesis of the neurotransmitters, dopamine, norepinephrine, epinephrine and serotonin in both the peripheral and central nervous systems, as well as from an impaired ability to hydroxylate phenylalanine in the liver. Indeed, to our knowledge, these patients are the only population presently available whose neurological dysfunctions can unequivocally be attributed to a genetic defect in biogenic monoamine synthesis which does not appear to involve irreversible cell loss. These patients might therefore be considered as models for other nondegenerative neurological diseases, the etiology of which is believed to involve aberrations in biogenic monoamine metabolism.

Current therapy for these variant forms of hyperphenylalaninemia consists of restriction of phenylalanine intake and administration of the hydroxylated amino acid precursors of catecholamines and serotonin, 3,4-dihydroxyphenylalanine (DOPA) and 5-hydroxytryptophan, respectively, in conjunction with inhibition of peripheral aromatic amino acid decarboxylation with carbidopa. Although administration of BH_4 to these patients, especially to those with a defect in BH_4 biosynthesis, might also appear to be a reasonable therapy, the reports that this pterin does not readily enter the brain from the periphery made it seem unlikely that this treatment would prevent the neurological damage that characterizes these diseases.

Major Findings:

We have previously shown that BH_4 and 6MPH₄, when given orally at a sufficiently high dose, can cross the blood brain barrier. We have continued to explore the use of tetrahydropterins in the treatment of hyperphenylalaninemia caused by defects in the de novo synthesis of BH_4 . In studying two children with this disease, we have found that BH_4 or 6MPH₄ administration given at the dose that we had established in our previous studies, namely 12 to 20 mg/kg/24 hours, led to marked clinical improvement in one of these patients and only modest improvement in the other one. This variable response does not appear to be caused by any difference in the ability of the pterins to cross the blood brain barrier in the patients since comparable amounts of the pterins were detected in samples of their CSF.

We have found that the administered 6MPH₄ very significantly elevated CSF levels of metabolites of dopamine and serotonin in the responsive patient, whereas there was no such effect in the unresponsive patient. These results indicate that there may be a correlation between the ability of 6MPH₄ or BH₄ to improve the neurological status of these patients and their ability to stimulate the cerebral synthesis of the mono-amine neurotransmitters. We have also found that the patient who showed only modest improvement when given tetrahydropterins, showed a more pronounced improvement when maintained on neurotransmitter precursors, DOPA and 5-hydroxytryptophan. By contrast, with the responsive patient, there are indications that pterin administration leads to greater improvement than does neurotransmitter precursor therapy.

Significance to Biomedical Research and Proposed Course of Project:

These results indicate that pterin therapy of diseases caused by defects in BH₄ synthesis may have to be initiated prenatally in order to achieve maximum benefits. Furthermore, the variable response to pterin therapy indicates that BH₄ may play a role in normal brain development, a role that is probably related to its essential cofactor role with tyrosine and tryptophan hydroxylases.

In order to explore such a developmental role, we are trying to develop inhibitors of one or more enzymes involved in BH₄ biosynthesis. Based on our clinical results, we intend to explore the possible use of a combined therapy involving the administration of both neurotransmitter precursors and tetrahydropterins. It is also possible that with such a combined therapy, the dose of tetrahydropterin needed can be significantly reduced. Since BH₄ is still extremely expensive, any therapy in which smaller amounts of BH₄ can be used would represent a significant advance.

We plan to apply what we have learned about the use of pterin administration in the treatment of PKU due to defective BH₄ synthesis to the treatment of other diseases where impaired synthesis of biogenic amines has been implicated, such as certain forms of depression.

References:

1. Kaufman, S., Kapatos, G., McInnes, R. R., Schulman, J. D., and Rizzo, W. B.: The use of tetrahydropterins in the treatment of hyperphenylalaninemia due to defective synthesis of tetrahydrobiopterin: Evidence that peripherally administered tetrahydropterins enter the brain. Pediatrics, **70**, 375-380, 1982.
2. Kaufman, S., Kapatos, G., Rizzo, W. B., Schulman, J. D., Tamarkin, L., and Van Loon, G. R.: Tetrahydropterin therapy of hyperphenylalaninemia due to defective synthesis of tetrahydrobiopterin. Annals of Neurology, 1983. In press.
3. Kaufman, S.: Phenylketonuria and its variants. Advances in Human Genetics, 1983. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01039-15 LNC
PERIOD COVERED October 1, 1982 through September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Pteridine Biosynthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Seymour Kaufman, Chief, Laboratory of Neurochemistry, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurochemistry		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0.0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <div style="border: 1px solid black; padding: 10px; min-height: 300px;"> <p>Several mechanisms have been found which appear to be involved in the regulation of tetrahydrobiopterin (BH₄) biosynthesis. In the adrenergic neuroblastoma cell line, NIE115, BH₄ synthesis was found to be inhibited by pterins including BH₄, folate and dihydrofolate. In rats, evidence was obtained which indicates that phenylalanine is capable of stimulating the synthesis of BH₄.</p> </div>		

OTHER PROFESSIONAL PERSONNEL:

Sheldon Milstien, Ph.D.

Research Chemist

LNC NIMH

Project Description:

Variant forms of PKU which result from a defect in tetrahydrobiopterin biosynthesis have been described. These children also suffer from neurological defects due to their inability to produce neurotransmitters derived from dihydroxyphenylalanine and 5-hydroxytryptophan, in support of the proposed physiological role of tetrahydrobiopterin in the hydroxylation of tyrosine and tryptophan. The discovery of two hyperphenylalaninemic children with a peripheral defect in tetrahydrobiopterin synthesis and with normal central biopterin and neurotransmitter levels has raised the possibility that there may be either two pathways for biopterin biosynthesis or different modes of regulation of the same pathway in the brain compared to the liver.

Major Findings:

Biopterin biosynthesis in the pineal gland: The developmental pattern of the appearance of both tetrahydrobiopterin and GTP-cyclohydrolase, the first and rate-limiting enzyme in biopterin biosynthesis, were studied in the rat pineal gland in whole brain. Changes in tetrahydrobiopterin levels corresponded with those of GTP-cyclohydrolase levels. In the brain there were two peaks of activity, one at 2 days before birth and the other 10 days after birth. By contrast, in the pineal gland, both biopterin content and GTP-cyclohydrolase activity were low prenatally and increased to nearly adult levels over 20-30 days. Rats that were superior cervical ganglionectomized shortly after birth did not show any alterations in the developmental appearance of tetrahydrobiopterin in the pineal gland, an indication that neural input is not required for the normal development of pineal tetrahydrobiopterin.

Neuroblastoma: Previous experiments demonstrated that the adrenergic neuroblastoma cell line N1E115 synthesized biopterin from guanosine triphosphate. Some intracellular mechanisms of regulation of tetrahydrobiopterin biosynthesis have been reported.

The addition of tetrahydrobiopterin to the medium decreased the conversion of ^{14}C -guanosine to both ^{14}C -neopterin and ^{14}C -biopterin in a concentration-dependent manner with half-maximal inhibition occurring at approximately 300 μM , or slightly more than twice the endogenous concentration of tetrahydrobiopterin in these cells. The inhibition of the labelling of the pterin pool was shown not to be due to a decrease in the specific activity of guanosine triphosphate nor to an increase in degradation or efflux rates. Both folic acid and dihydrofolic acid also inhibited the incorporation of label into neopterin and biopterin.

A partially purified fraction of GTP-cyclohydrolase from these cells was inhibited in a concentration-dependent manner by all of the pterins that inhibited biopterin biosynthesis in the intact cells. The inhibition of GTP-cyclohydrolase is thus the likely site of inhibition of tetrahydrobiopterin biosynthesis in the cells.

Biopterin and cell differentiation: There is a high concentration of tetrahydrobiopterin in reticulocytes which then decreases as maturation to erythrocytes occurs. Several pro-myelocytic leukemia cell lines have been established in culture which can be induced to terminally differentiate by treatment with agents such as dimethyl sulfoxide or retinoic acid. No changes in biopterin or neopterin levels were seen in these cells upon differentiation, suggesting that these pterins may not play any role in differentiation.

Regulation of tetrahydrobiopterin biosynthesis by phenylalanine: Treatment of rats with phenylalanine results in a dose-dependent increase in blood biopterin levels. The increase appears to be specific for L-phenylalanine, since no other amino acid tested had any effect on biopterin levels. Despite large increases in brain amino acid concentrations after intraperitoneal administration, no changes in brain biopterin concentrations were detected with any of the amino acids tested.

The rate of synthesis of tetrahydrobiopterin in the intact rat was studied by the administration of ^{14}C -guanosine to label intracellular guanosine triphosphate pools shortly before administration of the amino acids. Isolation of ^{14}C -biopterin from urine and determination of its specific activity demonstrated that L-phenylalanine increased the rate of conversion of ^{14}C -guanosine to biopterin. No other amino acid tested had any significant effect on biopterin specific activity, in agreement with the effect on blood biopterin levels.

Experiments are in progress to elucidate the site of action of phenylalanine.

Biopterin biosynthesis in rat brain and liver: The biosynthesis of tetrahydrobiopterin has been proposed by several groups to proceed through 7,8-dihydrobiopterin as an intermediate. The final step in the biosynthesis of tetrahydrobiopterin would then be a reduction catalyzed by dihydrofolate reductase. Recently, it has been reported that tetrahydrobiopterin in neural tissues is not sensitive to methotrexate inhibition, suggesting that dihydrofolate reductase, a methotrexate-sensitive enzyme, is not required for tetrahydrobiopterin biosynthesis (Nichol *et al.*, Proc. Nat. Acad. Sci. 80, 1546 (1983)).

Crude rat brain and liver extracts were fractionated on DEAE-cellulose and 7,8-dihydrobiopterin reductase activity determined in all of the fractions. Only a single, methotrexate-sensitive enzyme activity peak was found in both brain and liver. This activity co-eluted with the dihydrofolate reductase activity. Methotrexate titration curves of both the dihydrofolate and dihydrobiopterin reductase activities were identical. These results suggest that there is no previously undescribed methotrexate-insensitive 7,8-dihydrobiopterin reductase in either brain or liver. Moreover, these results indicate that if 7,8-dihydrobiopterin is an intermediate in tetrahydrobiopterin biosynthesis, the reductase must be inaccessible to methotrexate.

Significance to Biomedical Research and Proposed Course of Project:

Several *in vitro* systems have been devised to study the biosynthesis of tetrahydrobiopterin. The biosynthetic pathway in neurally-derived tissues has been

shown to be regulated by the end product, tetrahydrobiopterin, as well as by other pterins. Phenylalanine has been shown to increase biopterin biosynthesis in the periphery. Studies are in progress on the nature of the biosynthetic pathway and on the relationship between the regulation of tetrahydrobiopterin biosynthesis and the regulation of neurotransmitter levels. These studies should contribute to our understanding of the neuropathology in humans defective in tetrahydrobiopterin biosynthesis.

Publications:

1. Kapatos, G., Kaufman, S., Weller, J. L., and Klein, D. C.: The development of tetrahydrobiopterin and guanosine-5-triphosphate cyclohydrolase: differential patterns in rat brain and pineal gland. Brain Res. 258, 351-355, 1983.
2. Kapatos, G., Kaufman, S., Weller, J., and Klein, D. C.: Development and regulation of biopterin in the pineal gland of the rat. In Melatonin Rhythm Generating System, Ed. D. C. Klein, Karger AG, Basel, Switzerland, 1982, pp. 108-123.
3. Kapatos, G., Katoh, S., and Kaufman, S.: Biopterin biosynthesis by rat brain. J. Neurochemistry, 39, 1152-1162, 1982.
4. Kapatos, G., and Kaufman, S.: Inhibition of pterin biosynthesis in the adrenergic neuroblastoma N1E115 by tetrahydrobiopterin and folate. In: Chemistry and Biology of Pteridines, (Ed.) H.C.S. Wood. In press.
5. Milstien, S., and Kaufman, S.: The regulation of biopterin biosynthesis in the rat. In: Chemistry and Biology of Pteridines, (Ed.) H.C.S. Wood. In press.
6. Milstien, S., and Kaufman, S.: Dihydrofolate reductase catalyzes the reduction of 7,8-dihydrobiopterin in liver and brain. In: Biochemical and Clinical Aspects of Pteridines. (Ed.) H. Ch. Curtius. In press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01081-13 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (90 characters or less. Title must fit on one line between the borders.) Cerebral Control of Voluntary Movement		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) E. V. Evarts Chief, Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 4.00	PROFESSIONAL: 4.00	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 30%;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div style="width: 30%;"> <input type="checkbox"/> (b) Human tissues </div> <div style="width: 30%;"> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project utilizes <u>single neuron recording</u> and <u>operant conditioning</u> techniques in behaving <u>monkeys</u> to study <u>brain mechanisms</u> underlying voluntary movement. Monkeys are trained to make precise movements of a handle whose position controls a visual display, and <u>stimuli</u> are delivered via the handle by means of an electronically controlled <u>torque motor</u> in order to determine how <u>sensory feedback</u> is processed. Recordings from primary <u>sensory cortex</u> when movements are triggered by a vibrotactile stimulus show that central influences corollary with initiation of an active movement modify sensory cortex <u>information processing</u>. In a separate experiment on <u>basal ganglia</u> it has been found that there is a class of neurons exhibiting responsiveness when a stimulus serves as a cue for movement but not when the motor response to this same cue has been extinguished. Taken together, these studies provide new evidence for brain loci involved in control of <u>voluntary movement</u>. </p>		
(957)		

Project Description:

Cerebral control of voluntary movement depends upon the integration of internally generated central programs with peripheral inputs arising from sensory receptors. Understanding, treatment and prevention of movement disorders in neuropsychiatric patients requires knowledge of the sites at which this integration takes place. Two cerebral structures: 1) The Sensorimotor Cortex 2) The Basal Ganglia are of particular significance in control of voluntary movement. This project is aimed at discovering how these two structures interact. Work on the basal ganglia is of particular significance in connection with clinical disorders, since it provides techniques for discovering how toxic substances produce Parkinson's disease and how therapeutic agents exercise beneficial effects in Parkinson's disease. Work on volitionally-related activity in sensorimotor cortex provides information as to the way in which the central set and expectancy of the subject may modify processing of afferent input information and generation of output signals to spinal cord.

Work on basal ganglia is currently aimed at an analysis of the properties of cells located in the striatum, a part of the basal ganglia that receives inputs from many areas of cerebral cortex and relays its output through structures that project back to non-primary motor cortex (see LNP Project of Wise on "The Non-Primary Motor Cortex and the Cerebral Control of Movement").

Striatal processing of information from the cerebral cortex is critically dependent on the neurotransmitter dopamine, and the level of this neurotransmitter is regulated by a relatively small number of cells in a part of the basal ganglia called the substantia nigra (SN). A tract of fibers (the nigrostriatal tract) links SN and striatum and activity of cells in SN will cause release of dopamine in striatum. Parkinson's disease results from loss of SN cells and is accompanied by marked reductions of dopamine levels in striatum. It is not yet known how the activity of striatal cells is changed in parkinsonism, but the recent discovery that parkinsonism can be produced experimentally in the monkey by a drug (NMPTP) provides a model of the human neurological disorder in which this information can be obtained. The findings on nerve cell activity in striatum described below were obtained to provide information that would then be compared to results of comparable recordings of neuronal activity in monkeys with experimental NMPTP-induced parkinsonism. Other investigators on this project are Randall J. Nelson, Staff Fellow, Laboratory of Neurophysiology, NIMH; Janusz Rajkowski and Minoru Kimura, Visiting Fellows, Laboratory of Neurophysiology, NIMH.

Major Findings:

A primary focus in initial studies has been to characterize the properties of tonically active striatal cells whose discharge patterns may reflect dopamine levels in the striatum. It has been found that tonically active striatal cells may be divided into two classes on the basis of their discharge patterns and that these two classes are related to behavior in ways that are subtle but potentially important. Cells were initially picked up during limb and orofacial movements and then studied during a subsequent period of motor quiescence. Many neurons that were active with movement became virtually silent during absence of movement (average frequency less than 1 Hz). The activity of the units that

were not silent at rest fell into 3 categories, 2 of which had virtually continuous activity at between 3 and 7 Hz. In contrast, cells in the third category had relatively long periods of quiescence interrupted by occasional bursts. The first category of tonically discharging neurons (Type I) had a unimodal interspike interval distribution while the second category of tonic cells (Type II) exhibited a bimodal interspike interval distribution. Units that were for the most part silent but exhibited occasional bursts had interspike interval distributions with two modes, one of which was extremely short (corresponding to the intervals associated with the bursts) while the other was quite long, corresponding to the periods of silence. When units showing bursts and long periods of silence were carefully examined in relation to spontaneous movements occurring during periods that were otherwise quiescent, it was found that in fact their bursts were usually associated with slight spontaneous movements.

Tonically active striatal units classied as indicated above were studied in 3 behavioral paradigms: SELF-PACED MOVEMENT (SPM): flexion-extension of elbow for juice reward followed by licking. FREE REWARD (FR): rewards delivered with arm position fixed; monkey promptly relaxed the arm with onset of FR. NO REWARD (NR): similar to FR except that the tube conveying the juice was occluded so the solenoid click was no longer followed by reward and licking extinguished after the first few solenoid clicks in the block of 40 consecutive NR clicks. Tonically active units lacked intense discharge time-locked to movement, but type I tonic units often exhibited one or two impulses evoked at short-latency by the solenoid click preceding reward in SPM and FR, but not in NR, when click-triggered licks had extinguished. Type II units, (i.e., those with bimodal interspike interval distributions) rarely exhibited short-latency responses but did exhibit a synchronization of their tonic discharge following the solenoid click during SPM and FR. Synchronization also occurred in some type I units.

The existence of a class of tonically active striatal cells whose responses are contingent upon the behavioral significance of a sensory stimulus suggests the possibility that this class of cells may provide set-dependent links between input and output, and makes this class of cells of interest in the forthcoming evaluation of the effects of experimental parkinsonism on activity of striatal neurons.

A second phase of this project on cerebral control of voluntary movement has dealt with effects of volitionally initiated movement on somatosensory information processing in the cerebral cortex. In this phase of the project responses of somatosensory cortical neurons to peripheral stimuli were determined when a vibrotactile stimulus served as a cue for the initiation of an active movement executed with the stimulated extremity.

Rhesus monkeys flexed or extended the wrist in response to a vibrotactile cue (27-157 hz low amplitude sine wave) delivered via the manipulandum after they maintained a fixed forelimb position for a randomized hold time (0.5-1.5s). The stimulus remained on until the hand had moved 5 degrees around the wrist. Of a total of 644 task related cells recorded in the pre- and postcentral cortex, 143 postcentral cells were chosen for this analysis because: 1) each had a short latency response (<30 ms) to the stimulus onset, and 2) each showed further modulation related to some facet of the motor task. There was a marked decrease

in the firing rate within 30 ms after stimulus onset in 59 cells. Of these, 19% had cutaneous receptive fields RFs, 13% with RFs in contact with the manipulator and 6% with RFs located on other parts of the forelimb. Some 71% had well defined responses to joint manipulation. A few (10%) were sensitive to peripheral stimulation but no cutaneous field could be defined and were classified as having deep input. In no instance was there a decrease in firing rate in these same cells when a tactile stimulus was applied while the animal sat passively. A total of 84 cells responded to the vibrotactile cue with increased discharge within 30ms of stimulus onset, including 30 cells which were entrained to the stimulus frequency. This population includes 42% which had proprioceptive responses, 32% had cutaneous RFs, 14% had deep input and 12% were lost before testing. Of these 84 cells which exhibited increased discharge with the stimulus, 60% had a profound decrease in firing rate approximately 60-80ms prior to earliest detectable change in handle position. This decrease corresponds to the time at which neurons in precentral cortex exhibit peak activity during this task.

These observations suggested that cells in somatic sensory cortex are subjected to at least two types of influences. The first occurs just after stimulus onset and is present only preceding movement of the hand. The second occurs at approximately the same time as peak precentral cortex. Both types of influences may have important central, as opposed to peripheral, components as evidenced by their dependence on and correlation with active movement.

Proposed Course:

Studies on basal ganglia will be directed to discovering the alterations of nerve cell discharge occurring in an experimental primate model of parkinsonism. Preliminary findings have already been obtained in one monkey treated with NMPTP, and within the coming year it should be possible to obtain sufficient data to reach firm conclusions as to the alterations of striatal activity in experimental parkinsonism and the way in which L-DOPA acts to alleviate movement disorders.

The studies on sensorimotor cortex information processing during voluntary movement will be extended to an investigation of the pathways that may underlie the changes of sensorimotor neuron responses to afferent input. In particular, we will seek to identify the sites causing attenuation of sensory inputs at the time of voluntary movement initiation.

Significance to Biomedical Research and to the Program of the Institute:

This project seeks to apply basic research results on central control of voluntary movement to an understanding of normal and abnormal movements in man. To the extent that the project succeeds, it will contribute to the research goals of the NIMH. Thus far, the project has shown that the laws of reflex action, long known to operate at the level of the spinal cord motoneuron, also operate at the level of the cerebral cortex in the course of volitional movements. Motor cortex neurons are impinged upon by afferent inputs which constitute the incoming limb of a transcortical servo loop. Thus, the phylogenetically new motor cortex of the mammal is subject to the same laws of reflex action that characterize phylogenetically older components of motor control systems. But in addition to being driven by a servo system which stabilizes movement

and posture, motor cortex can be driven by a second major set of inputs, and it is this second set of inputs that underlies internally generated motor programs. These programs, reaching the motor cortex from the thalamus, are themselves a product of activity in red nucleus, basal ganglia, and cerebellum. Within the past year this project has begun a new and exciting phase involving a combined neuropharmacological-behavioral investigation of cells that depend on a dopaminergic input from the substantia nigra. Anti-parkinsonian agents such as L-DOPA act on these cells, and the availability of a new primate model of parkinsonism opens a number of new approaches to understanding the effects of dopamine in the brain. Such information is clearly relevant to the research program of the NIMH, since a major feature of many neuroleptic agents is antagonism to dopamine.

Publications:

Evarts, E.V.: Pyramidal tract neurons and mechanisms for recovery of function following lesions of motor cortex. In Buser, P.A., Cobb, W.A. and Okuma, T. (Eds.): Kyoto Symposia (EEG Suppl No. 36). Elsevier Biomedical Press, Amsterdam, 1982, pp. 147-152.

Evarts, E.V.: Control of voluntary movement by the brain: Contrasting roles of sensorimotor cortex, basal ganglia and cerebellum. In Buser, P.A., Cobb, W.A. and Okuma, T. (Eds.): Kyoto Symposia (EEG Suppl No. 36). Elsevier Biomedical Press, Amsterdam, 1982, pp. 385-392.

Evarts, E.V., Fromm, C., Kröllner, J. and Jennings, V.A.: Motor cortex control of finely-graded forces. J. Neurophysiol. 49: 1199-1215, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01090-07 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Central Nervous System Functional Anatomy		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Miles Herkenham Research Psychologist Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any) The Clinical Neuroscience Branch		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 3.25	PROFESSIONAL: 3.25	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A sensitive method for <u>light microscopic localization of brain receptors by in vitro autoradiography</u> was developed previously in this laboratory. By this method we have mapped the <u>locations of opiate receptors</u> in the brains of rats and other vertebrates, including <u>primates</u>. Comparisons of tritiated <u>naloxone</u> binding with tritiated <u>enkephalin</u> binding have reinforced the notion of <u>opiate receptor subtypes</u>. These have been followed <u>ontogenetically and phylogenetically</u> and have been related to the dopamine system in the striatum. The possibility of <u>pharmacological manipulation of receptor distribution</u> is being examined. Applications are being pursued for the study of receptors and <u>biologically active peptides and enzymes</u> in <u>unfixed human brains</u> obtained at autopsy. The unfixed, cryostat-cut tissue is amenable for concurrent study of <u>metabolic and functional mapping by 2-deoxy-D-glucose</u>. For example, we have compared <u>phencyclidine (PCP) receptor</u> localization patterns with patterns of altered brain metabolic activity produced by <u>phencyclidine "anesthesia"</u>. This technique has been utilized in functional studies of the <u>extra-pyramidal motor system</u>, regions which influence <u>sexual behavior</u>, and the neuropharmacologic and hormonal manipulation of these systems. </p>		

I. Studies of CNS Functional Neuroanatomy. Neurochemical Investigations.

Objectives:

For the past four years, a collaborative effort with Dr. Candace Pert in the Clinical Neuroscience Branch of the NIMH has been directed toward localizing the brain sites of action of pharmacologically active drugs and putative neurotransmitters. The discovery by Dr. Pert and others in 1973 of brain receptors for opiates and opioid peptides, and the subsequent localization of these receptors by neuroanatomical techniques, has opened up an important new area of functional neuroanatomy. The task of finding the neuronal circuitry that is "plugged into" these receptor sites requires knowing precisely both the distribution of the receptors and terminal distributions of fiber pathways in any given region. By such analysis we can identify the neuronal systems that contain the relevant transmitter and thereby make predictions about the neurochemical's role in normal brain function. Determinations of the locations and densities of the receptors in relevant brain regions can be used in tests of receptor changes during development, after chronic pharmacological manipulation, or in neuropathological tissues. Other investigators on this project are Sandra Moon Edley and Ronald P. Hammer, Jr., Staff Fellows in the Laboratory of Neurophysiology, NIMH.

Methods Employed:

We have successfully developed an in vitro autoradiographic technique for visualizing drug and neurotransmitter receptors in slide-mounted tissue slices. Fresh frozen cryostat-cut brain sections are securely attached to glass slides by a process of thaw-mounting and subsequent drying at cold temperatures. Slides are then incubated in solutions containing radiolabeled ligands. Excess and non-specifically bound ligand is washed off in cold buffered rinses, and the slides are blown dry. The sections are fixed in hot paraformaldehyde vapors under a vacuum, defatted in xylene and alcohol rinses, dried and then dipped in radioactive-sensitive emulsion for autoradiography. Alternatively, fixed sections can be placed in an x-ray cassette and overlain with LKB tritium-sensitive film. The developed film autoradiogram then can be analyzed by a densitometer for computer-assisted quantification of receptor densities. While emulsion-coated sections provide high resolution analysis through the microscope, films can be computer-analyzed for rapid quantification of receptor densities or for color-coded image enhancement.

Major Findings:

The method we developed for in vitro autoradiographic localization of brain receptors has been published. Most of our independent and collaborative efforts have been in the area of opiate receptor localization. An important contribution of the study of anatomical locations towards understanding the molecular biochemistry of opiate receptor subtypes has been the demonstration that ligand binding conditions can be optimized to reveal similar receptor distribution patterns, even when opiate alkaloids, peptides or other analogs are used as ligands. The autoradiography together with appropriate pharmacology performed on these same sections supported the hypothesis that the high affinity forms of the mu,

delta and kappa subtypes are different conformations of a single, interconvertible, dynamic receptor. Studies of the development of opiate receptors and related neurochemical systems are currently in progress, in an attempt to better understand the role that receptors play in the establishment of neuronal connections. Opiate receptors undergo phylogenetic changes as well, as suggested by our finding that the ratio of mu to delta receptor binding increases in parallel with "relatedness to humans" (as indicated by cytochrome C analysis of several vertebrate species). Analysis of mu opiate receptor distributions in rat and rhesus monkey cerebral cortex has revealed several general principles; e.g., opiate receptor density is greatest in limbic and polysensory (association) cortices.

These findings, taken together, suggest a role for opiates in brain function that is much more complex than previously thought and indicate that further analysis of the dynamic aspects of the receptor, after pharmacological or behavioral manipulations, might enhance our understanding of its function. Ultimately, we might hope to determine the role that opiates and related neurochemicals play in human brain function, especially in receptor-mediated mental disorders or neuropathology.

II. Metabolic Correlates of Functional Activity

Objectives:

We have developed high-resolution autoradiographic techniques for the localization of metabolic activity at the light microscopic level. Patterns of metabolic activity marked by [^3H]2-deoxyglucose uptake have been compared in normal, alert rats to those of animals given various drugs. Using series of adjacent tissue sections from a single animal, patterns of metabolic activity during drug administration can be correlated with the localization of receptors to which the drug binds.

Methods Employed:

We have developed techniques which permit us to use [^3H]2-deoxy-D-glucose (2-DG) as a metabolic marker of glucose utilization, visible at the cellular level of resolution. Low resolution was a persistent problem in previous autoradiographic localization studies, which utilized [^{14}C]2-DG as a marker. The substitution of [^3H]2-DG improves resolution, since the particles of [^3H] are less energetic than those of [^{14}C] and form an image closer to their source.

Visualization of metabolic activity of individual neurons requires fixation of the diffusible 2-DG molecule to its uptake site. This may be accomplished by the fixation of 2-DG *in situ* using perfusion with a light-to-medium strength paraformaldehyde fixative followed by cryosectioning and mounting. After perfusion, 2-DG and its labeled metabolites are retained in brain tissue. Moreover, labeling of cellular regions is enhanced. Current evidence suggests that some of the 2-DG may be incorporated into intracellular glycogen, which is fixed in place during perfusion and does not diffuse from its cytoplasmic location during later phases of aqueous processing. Alternatively, these

sections can be quickly dried and exposed to tritium-sensitive film to retain in place the labeled diffusible 2-DG-6-phosphate, which is the major 2-DG breakdown product. Increased resolution of [^3H]2-DG autoradiographs is also due to the quenching of ^3H -particle emission by white matter, resulting in greater contrast between brain regions of differential white matter content. This factor facilitates visualization of cellular regions surrounded by fiber-rich regions.

Autoradiographic localization of brain receptors can be compared in the same animal with manipulation-induced alterations in brain metabolism measured by the 2-DG technique. Alternate sections from an animal previously injected with 2-DG are either processed for 2-DG autoradiography as described or for receptor localization. The latter is accomplished by first removing the diffusible 2-DG in preincubation solutions prior to in vitro receptor binding. In this way, the alteration of brain metabolism by drugs or anesthetics may be correlated with receptor binding in those brain regions affected.

Major Findings:

Our recent studies of phencyclidine-induced changes in brain metabolism elucidate the mechanism of action of phencyclidine analogs in the central nervous system. Metabolism in regions of limbic cortex is stimulated by these drugs while sensory cortical zones show decreased metabolic activity. This inhibition of glucose uptake occurs in all layers except layer Va of primary somatosensory and primary visual cortex; however, activity in secondary cortical regions is spared. Correlative 2-DG and receptor binding studies have shown that the cortical regions which show decreased metabolism also contain higher densities of GABA receptors. A phencyclidine-induced GABAergic influence on these specific cortical zones may be a factor in the decline of cortical sensory metabolism.

We have begun to investigate the functional role of monoamines as neurotransmitters in the extrapyramidal motor system using 2-DG techniques. Depletion of monoamine input to the striatum induced by chronic reserpine administration causes a metabolic activation of the globus pallidus, which receives afferent projections from the striatum. Reserpine depletes dopamine in the striatum and alters neuronal activity in both the striatum and pallidum. The disinhibition of pallidal activity leads to increased metabolism at this locus. These results suggest that dopamine directly influences striatal outflow; furthermore distant targets are functionally affected. Correlation of the sites of these metabolic changes with the location of dopamine receptors in the basal ganglia may further elucidate this process.

Significance to Biomedical Research and to the Program of the Institute, and Proposed Course:

The visualization by autoradiographic techniques of opiate receptor locations throughout the CNS has greatly advanced our appreciation of the richness of opiate functions in normal physiology and has opened a surprising number of doors to the investigation of receptor-mediated brain processes. We have

just begun to appreciate how receptors influence and control neuronal development and the establishment of neural connections, the interrelatedness of receptor subtypes and of neurochemically distinct systems (such as dopaminergic and opiate interactions in the striatum), the evolution of receptors as markers of synaptic complexity and the significance of species differences. We are encouraged by comparisons of drug receptors and the altered metabolic profile (as seen by 2-deoxyglucose autoradiography) produced by the same drugs. These findings indicate a productive future in the research of brain function. Some preliminary data on receptor differences, visualized by autoradiography in genetically "nervous" dogs, may be of clinical importance.

Since these differences appear to be pharmacological, identification of correlates in human mental disorders would encourage us to analyze receptor distributions in the brains of deceased humans with histories of Parkinson's disease, Huntington's chorea and schizophrenia.

Publications:

Hammer, R.P., Jr.: A brain for all seasons. Trends in Neurosci., 5: 378, 1982.

Herkenham, M.: Autoradiographic demonstration of receptor distributions. In Baker, J.L. (Organizer): Strategies for Studying the Roles of Peptides in Neuronal Function. Short Course Syllabus. Bethesda, Maryland, Soc. Neuroscience, 1982, pp. 41-59.

Herkenham, M. and Pert C.B.: Light microscopic localization of brain opiate receptors: a general autoradiographic method which preserves tissue quality. J. Neuroscience, 2: 1129-1149, 1982.

Lewis, M.E., Patel, J., Moon Edley, S., and Marangos P.: Autoradiographic visualization of rat brain adenosine receptors using N6 Cyclohexyl 3H adenosine. Eur. J. Pharmac. 73: 109-111, 1981.

Moon Edley, S., Hall, L., Herkenham, M., and Pert, C.B.: Evolution of striatal opiate receptors. Brain Research, 249: 184-188, 1982.

Quirion, R., Bowen, W., Herkenham, M., and Pert, C.B.: Visualization and solubilization of rat brain opiate receptors with a " " ligand selectivity pattern. Cell. Mol. Neurobiol., 2: 333-346, 1983.

Quirion, R., Hammer, R.P., Herkenham, M., and Pert, C.B.: Autoradiographic localization of the phencyclidine/sigma "opiate" receptor in rat brain. In Harris, L.S. (Ed.) NIDA Res. Monograph No. 41, Washington, D. C., 1982, pp. 178-183.

Wise, S.P., and Herkenham, M.: Opiate receptor distribution in the cerebral cortex of the rhesus monkey. Science, 218: 387-388, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01091-06 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Motor Function in Patients with Neuropsychiatric Disorders		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Jerome N. Sanes Staff Fellow Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any) Experimental Therapeutics Branch, NINCDS		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purposes of this project are to examine the contributions of central <u>motor programming</u> and <u>afferent input</u> in control of <u>arm movements</u> in normal subjects and patients with <u>sensori-motor disorders</u>, and to study <u>psychomotor performance</u> of patients with central motor disorders. The first set of experiments records <u>muscle activity</u> and <u>kinematics of limb position</u> while (1) subjects manually match a target display with either a skilled rapid or slow movement with a handle whose displacement controls a visual display or (2) maintain <u>postures</u> when limb position is passively changed. <u>Movement amplitude</u>, presence or absence of <u>visual feedback</u> of position, disturbances of the subject's movements and changes in sensory input are independent variables. <u>Large movements</u> are performed accurately independent of manipulation of the experimental variables but accurate performance of <u>small movements</u> becomes increasingly dependent on the absence of limb disturbances during movement. The second set of studies examined a variety of psychomotor variables from patients with a variety of neurological disorders. Voluntary and involuntary movements are evaluated to develop sensitive measures of psychomotor performance that correlated with clinically determined fluctuations in <u>drug efficacy</u>. </p>		

I. Central and Peripheral Control of Movement in Humans.

Project Description:

The importance of afferent information in the control of limb movements is controversial. Whereas it is clear that afferents exert potent physiological effects on spinal motoneurons and cells in supraspinal structures, it has been suggested that some of these afferents contribute little to the final positioning of a limb. A case in point is the observation that muscle spindle activity does not reflect muscle length during rapid movements of large amplitude, thereby casting doubt on a regulatory role for spindles at the end of movements. In addition, physical disturbances imposed during movements, that likely activate muscle spindles, do not appear to modify final limb positioning. There are, however, other experiments demonstrating the importance of afferent input in a variety of tasks performed by humans. For example, ischemic deafferentation of limbs alters position sense and sense of effort. Furthermore, performance of fine motor tasks, such as reproduction of alphabetic characters is also disrupted by ischemic deafferentation and it is noteworthy that inactivation of the gamma loop in humans impaired the ability to tonically activate motor units, though phasic activation was not impaired. It is the object of the present project to continue examination of the role of peripheral inputs in the control of limb movements and postural control. Both normal volunteers and patients with neurological disorders will be studied during performance of movements of varying sizes and when a maintained posture is disturbed by different peripheral inputs. Different types of limb disturbances will be imposed during the movements. Two general experimental approaches are being pursued. In the first, the psychomotor variables of movement error and movement time are studied in relation to physical disturbances. In the second group of experiments, electromyographic activity is examined when the limb is mechanically perturbed while subjects perform motor tasks or maintain postures. Other investigators on this project are Edward V. Evarts, Chief, Laboratory of Neurophysiology, NIMH; Karl-Heinz Mauritz, Guest Worker, Laboratory of Neurophysiology, NIMH; Von A. Jennings, Staff Fellow, Laboratory of Neurophysiology, NIMH and Peter LeWitt, Clinical Associate, Experimental Therapeutics Branch, NINCDS.

Methods:

Human subjects are trained to manipulate a handle that is attached to a servo-controlled torque motor while performing extension-flexion of the wrist, elbow or index finger, or abduction-adduction of the index finger. Displacement of the handle causes movement of an oscilloscope beam that is to be matched by the subject with a second, experimenter controlled, oscilloscope beam. In one series of experiments, subjects perform tracking movements either as rapidly as possible or as accurately as possible. For a variety of movement sizes (3° to 30°) subjects are given an adequate number of training trials. Independent variables include (1) continuous loads opposing or assisting movement, (2) brief physical disturbances delivered to the arm before or after initiation of arm movement and (3) initial starting position. Patterns of muscle activity and tracking errors are analyzed during rapid movements.

Preliminary Findings:

Motor behavior of normal subjects and patients with large fiber sensory neuropathy has been studied. Several findings have emerged:

- (1) In normal subjects, unexpected presentation of viscous loads at the onset of movement increases error of limb positioning. This effect increases as movement size decreases, so that, for example, the error is increased by 40% and 10% for 3° and 30° movements, respectively, when a viscous load opposes movement. A similar result was obtained when a viscous load was unexpectedly removed at the onset of movement. Muscle responses to the unexpected increase or decrease in viscosity occur at a short latency of about 50 msec.
 - (2) In patients with sensory neuropathy, the unexpected presentation of a viscous load results in undershooting of the final position. Since these patients cannot detect the change in viscosity there were no compensatory muscle responses to overcome increases in mechanical resistance. A similar result occurs when the movements of patients with sensory neuropathy are briefly stopped and do not compensate after the mechanical obstruction has been removed.
 - (3) The sense of effort was evaluated in normal subject and patients with sensory neuropathy. Four methods were used, all using either classical or modern methods typically employed in sensory detection tasks. Subjects were required to detect the difference in constant torques that opposed hand movement. In unimanual detection tasks, subjects either attempted to maintain a steady position against several different opposing torques or moved the hand 45° against different opposing torques. Subjects evaluated the effort required to maintain posture or move on each trial compared to the previous trial. The loads were changed according to the Method of Constant Stimuli and the data were analyzed with the Methods of Signal Detection. In the bimanual detection tasks, one of five constant torques continuously opposed movement of the left hand while for each trial loads were changed for the right hand. Subjects compared the effort required to maintain both hands stable. The loads to the right hand were changed according to the Methods of Constant Stimuli and the Method of Limits.
- On all tasks, patients were impaired in the ability to correctly compare opposing torques. Most errors occurred with torques that were not very different, but patients made incorrect decisions even when torques were substantially different.
- (4) The development of fatigue tremor in patients with sensory neuropathy and controls was evaluated. Subjects extended the arm for 10 minutes while records of involuntary movements were taken every minute. A tremor at 8-12 Hz developed in the control subjects after about 5 minutes and continued to increase in amplitude until the test was stopped. In contrast, patients did not develop any rhythmical involuntary movements but instead showed increases in all frequencies of movement.
 - (5) The ability of patients with sensory neuropathy to maintain stability against an unstable load was grossly impaired. Patients were required to move the hand to a new position against a spring load that worked to push hand back to initial position. Patients were able to do this task when visual guidance of

hand position was available. In contrast, when memory of motor output was required (i.e. without visual guidance) patients drifted away from the intended position. In addition, the drift typically was coincident with removal of visual guidance. The muscle activity pattern accompanying the abnormal postural response was abnormal in that (1) co-contraction, (2) dissipation of agonist muscle activity and (3) alternate activation and quiescence of antagonist were observed on individual trials. Normal subjects perform this task identically independent of the conditions of visual guidance.

II. Objective Psychomotor Evaluation of Neuropsychiatric Patients

Project Description:

In the last four annual reports we described the technical aspects and the use of a computerized system for obtaining objective, quantitative measures of motor function in patients with neurological disorders. Following the partial completion of the system in the summer of 1979, the measurement apparatus was installed in an appropriate setting for use in testing patients with neurological disorders in the Clinical Center. The system has been used to record and subsequently summarize data concerning motor functions in more than 100 patients with parkinsonism participating in clinical trials of the experimental anti-parkinsonian drugs, lisuride, bromocriptine, pergolide and nadolol. Patients with cerebellar disorders and essential tremor have also been studied. To establish normative data for movement variables measured on our system, thirty age-matched normal subjects have also been tested. An additional use of the computer system has been to begin studies of psychomotor performance using the methods devised by experimental psychologists. These studies will be useful to provide objective measures of the organization of motor performance and to assist the clinician in diagnosis and evaluation of patients with sensorimotor disorders.

Major Findings:

Studies of age-matched normals revealed a considerable range of responses in the parameters measured by the system, with values varying between individuals as a result of such factors as physical stature, motivation, and temperament. Individual scores, however, tended to be relatively consistent over time. Such expected outcomes as diminished speed of movement among members of the oldest age group when compared to younger normal subjects were observed. Parkinsonian patients undergoing treatment with lisuride, bromocriptine or pergolide were tested on a regular basis throughout the course of the build-up and placebo phases of the clinical trial. Grouped data, according to age, confirmed clinical observations in assessing motor performance in individuals undergoing treatment or evaluation. Immediate evaluation of patient performance is available to the test administrators at the completion of each test and periodic summaries of performance may be prepared easily. Furthermore, data collected in all studies to date have been permanently stored in computer files and are accessible for further study. These features of the system could be valuable for longitudinal studies of patients with neuropsychiatric disorders.

A recent application of computer evaluation of psychomotor functions of patients with neuropsychiatric diseases has been the evaluation of tremor in cerebellar

disease. Of concern were the mechanical, physiological and psychological variables that affect tremor presented by patients with cerebellar disease. We studied patients with classical symptoms of intention tremor. The disorders were seen in patients with infarcts or diffuse olivo-ponto-cerebellar degeneration. We found that cerebellar intention tremor was influenced by the presence or absence of visual guidance: tremor was exacerbated for visually guided movements as compared to performance of the identical movements or postural maintenance without visual guidance. Mechanical factors also alter cerebellar tremor. Viscoous and inertial loads diminish the tremor, with very small viscosities and inertial loads providing a disproportionate amount of tremor reduction. Loads that activate extensor muscle groups exacerbate tremor while tremor is diminished by activation of flexor muscles.

Significance to Biomedical Research and to the Program of the Institute:

Additional clarification of how somatosensory information is used to control skilled motor activity is essential to the understanding of normal and abnormal motor behavior in humans.

Furthermore, these studies will provide standards of normal motor function and allow comparisons with patients with motor disorders to evaluate subclinical deficits and the efficacy of pharmacotherapeutic agents. The objective evaluation of neuropsychiatric disorders that we have developed should prove useful in a wide variety of experimental applications that require computer recording and analyses of results. Long-term evaluation of patients' progress on medication regimens is particularly suited for objective analysis.

Proposed Course:

Future studies concerned with psychomotor performance will continue to investigate the importance of tactile and kinesthetic signals occurring during movements. Thus, properties of movements and muscle activity will be investigated in normal and fatigued muscles, from functionally deafferented limbs, and following vibratory or mechanical disturbances delivered to a limb. A variety of movement types (e.g. large/small) and strategies (e.g. fast/slow) will be studied to determine the movements that depend upon sensory inputs from the periphery for accurate completion. More than two years of experimentation will be required to validate and extend the preliminary findings in providing additional information on the control of limb movements in normal and neurologically diseased humans.

The computer evaluation of movement is an ongoing project to provide objective evaluation of motor function in patients with movement disorders. Presently the neuroevaluation system is being used to continue evaluation of neuropsychiatric patients' movements when the accuracy and movement extent are varied. In addition, a new project that will study reaction and movement time during movement sequences will be initiated. The purpose of this study is to determine, objectively, the deficits in retrieval and execution of motor commands by patients with motor disorders.

Publications:

Ward, C.D., Sanes, J.N., Dambrosia, J.M., and Calne, D.B.: Methods for evaluating treatment in Parkinson's disease. In Fahn, S., Shoulson, I. and Calne, D.B. (Eds.): Experimental Therapeutics of Movement Disorders. New York, Raven Press, 1983. pp. 1-7.

Sanes, J.N. and Evarts, E.V.: Effects of perturbations on accuracy of arm movements. J. Neuroscience, 3: 977-986, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01092-05 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) The Non-Primary Motor Cortex and the Cerebral Control of Movement		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Steven P. Wise Research Biologist Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER:
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This project grew out of our neurophysiological and neuroanatomical investigation of the corticocortical connections in the <u>somatic sensorimotor cortex</u> of the monkey and their role in the control of <u>primate motor behavior</u>. In that project we redefined three of the main cortical inputs to the <u>primary motor cortex</u> (MI): the <u>premotor cortex</u> (PM), the <u>supplementary motor cortex</u> (MII) and the transition zone between the motor and somatic sensory cortex, area 3a. These three cortical fields surround MI and can be differentiated from MI on the basis of neuronal responses to peripheral inputs, thresholds for evoking movements with intracortical electrical stimulation, the properties of single neurons during the performance of an operantly conditioned motor task, cyto-architecture, and connectivity. In the past year, we have elaborated this study by an analysis of the activity of single neurons in PM during a visually guided motor task. These results support the hypothesis that PM plays a role in the execution of visually guided movements and the preparation for voluntary movements. </p>		

Objectives:

The inputs to the precentral motor cortex (MI) and its intrinsic neuronal circuitry determine the output of MI neurons, including those projecting to the spinal cord. We hope to gain an understanding of the afferent inputs to MI cortex and their interaction in producing motor cortex output. The long-term objective of this project is to examine the activity of neurons that project into and out of MI and to contrast the functional significance of corticocortical and corticofugal neurons. The other investigator on this project is Karl-Heinz Mauritz a Visiting Scientist at the Laboratory of Neurophysiology, NIMH.

Two more general objectives of this project are (1) an improved understanding of the organization of the entire motor cortex, a region which is likely to include, in addition to its "core," the MI cortex, a surrounding neocortical "belt" containing two or more representations of the motor periphery and (2) a better understanding of the cortical fields involved in the sensory guidance of movements and the linkage between sensory signals and motor behavior.

Methods:

Monkeys have been trained to perform several visually guided motor tasks. (1) A rhesus monkey was operantly conditioned to depress one of four keys located in a perimeter at arms length. While the monkey pressed one key, another of the four keys, selected randomly, was illuminated after a randomly varied delay. This key thereby became the next target. A barely discernable visual cue near the target key, appearing after another variable delay, signaled the monkey to move and depress the target. The monkey was required to make the movement within a short period of time, near the limit of reaction time. Neurons in the premotor cortex were studied in this experiment. (2) The monkeys were conditioned to align two spots of light on a tangent screen in front of the monkey. One of these spots is controlled by the computer (target), the other by arm movements of the animal (position). He was required to align the spots within a small accuracy "window." After a short period of time the target light jumped to one of six locations. The monkey had to maintain his arm position unchanged until the target light dimmed, at which point he was required to flex or extend his forearm rapidly and accurately. In one-sixth of the trials, the computer selected a situation in which physically identical stimuli signaled the animal to make no movement. This experiment was designed to contrast neuronal activity in MI and premotor cortex and to distinguish neuronal activity when identical stimuli signal the execution or withholding of movement. (3) The monkey was conditioned to depress a central key of three keys located in a perimeter in front of him. After a period of time, either the left or right key became illuminated. Three experimental conditions ensued: (a) the key remained illuminated and served as the target for the subsequently triggered movement, (b) the light was turned off before the monkey was allowed to execute the movement, forcing the monkey to remember the proper target, or (c) the target light was switched before the monkey was allowed to execute the movement. This experiment was designed to further test the relationship of neurons in MI and PM to the motor set of the animal, even when the signals are absent or the motor set changes during the course of a trial.

The single unit activity and behavioral data were collected on-line with a PDP 11/03 computer and analyzed off-line with a PDP-11/34 computer. Many of the routines used in off-line data analysis were developed by W. Sheriff of the Research Services Branch).

The past year has been devoted to conducting experiment #3, writing two reports of the results of experiment #2, finishing publication of the data obtained in experiment #1, and designing follow-up experiments. These next experiments, designed to explore the neural correlates of motor planning in premotor cortex and the topographic organization of premotor cortex, are in the developmental phases.

Following the recording procedures, small amounts (5-10 μ Ci) of [3 H]-amino acid can be injected into either the premotor cortex, MI or MII. By noting the ultimate distribution of radioactivity in the brain, the sites of termination of neurons in the somatic sensorimotor cortex can be determined by tissue autoradiography.

Experimental Findings:

About 800 units have been studied in four monkeys examined in this project to date. Several findings and interpretations have been developed:

1. MII and premotor cortex neurons are virtually unresponsive to peripheral somatosensory inputs, compared with MI neurons in the same monkeys. This finding is somewhat surprising from a neuroanatomical perspective, since MII receives monosynaptic corticocortical input from most subdivisions of the somatic sensorimotor cortex and premotor cortex has a variety of potential somatosensory inputs from cortical regions. However, the lack of profound somatic sensory responsiveness supports the hypothesis that MII and premotor cortex play a role in centrally generated motor programs rather than movements regulated by peripheral feedback.
2. These and additional findings have enabled us to improve the current understanding of cerebral localization in this part of the cortex, notably the relationship of physiologically defined cortical regions to those defined by anatomical methods. Two of these points are most noteworthy: (1) Microelectrode methods reveal that the boundary between MI and MII corresponds to the boundary between two anatomically defined parts of the agranular neocortex (termed areas 4 and 6 by Brodmann in 1909). The boundary between MI and premotor cortex corresponds not with the boundary between areas 4 and 6 drawn by Brodmann (1909) but rather an analogous boundary of von Bonin and Bailey (1947). (2) Area 3a, the transitional field between the agranular and the highly granular somatic sensorimotor cortex, appears to be, as it was originally defined (in the work of C. Vogt and O. Vogt, 1919) a discrete cortical field characterized by a thin internal granular layer (layer IV).
3. Our study of premotor cortex has shown that most neurons in that cortical field change activity markedly before the onset of a voluntary movement. Their activity is often specific for the direction of arm movement. These neurons are located within the frontal agranular cortex, corresponding to a part of area 6 as defined by the absence of a large population of giant, layer V pyramidal cells in addition to the lack of an internal granular layer (layer IV).

The premotor cortex can also be distinguished from the MI representation by its markedly increased threshold for evoking movements with intracortical microstimulation. Further, a substantial population of neurons change their activity in relation to motor set and/or signals which indicate the location of motor targets. One class of cell in premotor cortex, termed "set-related neurons," appear to be specifically correlated with the motor planning (or set) of the animal. This hypothesis has been supported in three ways: (a) these units show changes in activity when visual signals cue a movement, (thus establishing a specific motor set), but not when the same signals instruct the monkey to withhold movement, (b) when the guiding visual signal changes (thus changing the motor set), the unit activity changes to reflect the new presumed motor set, and (c) when the guiding signal is removed (but the set remains the same), the unit activity continues to reflect the motor set rather than the sensory signals.

Significance to Biomedical Research and to the Program of the Institute:

The activity of higher-order motor cortical fields such as premotor cortex is important to the understanding of the cortical control of motor acts of the least automatic kind, in both health and disease and especially for understanding the way in which sensory signals are converted, by the brain, into organized motor acts.

Proposed Course: In order to further examine the function of cortical connections, we must acquire more knowledge about the input-output organization of the cortex and the differential roles of the various fields within the somatic sensorimotor cortex especially those involved in higher-order control of movement. There is also a need for more reliable methods with which to distinguish the cortical fields from each other, especially in awake behaving animals. This project will be continued and developed in the Laboratory of Neurophysiology with the collaboration of Dr. K.-H. Mauritz and Dr. K. Kurata, a Fogarty fellow expected in the laboratory within the next year.

Publications:

Weinrich, M. and Wise, S.P.: The premotor cortex of the monkey. J. Neuroscience 2: 1329-1345, 1982.

Wise, S.P., Weinrich, M., and Mauritz, K.-H.: Motor aspects of cue-related neuronal activity in premotor cortex of the rhesus monkey. Brain Research 260: 301-305, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01093-05 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of Somatic Sensory Inputs in the Cerebral Control of Movements		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Von Jennings Staff Fellow Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) This project is a study of the role of <u>somatic sensory inputs</u> to the <u>cerebral cortex</u> in the <u>control</u> of <u>motor behavior</u> in <u>primates</u> . The first part of the project consists of a comparison of neural activity in the <u>somatic sensory cortex</u> (SI) and the <u>precentral motor cortex</u> (MI) with patterns of muscle activity during voluntary limb movements. The second part of the project is a detailed examination of the signals which the peripheral receptors are sending to the cortex during perturbation of voluntary movements. Such information should provide important clues concerning the pathway taken by peripheral inputs to MI and its role in the initiation and control of movement.		

Objectives:

Though peripheral inputs have been shown to affect MI neurons during movement, the significance and source of these inputs remains unclear. A possible role of sensory inputs to MI may be to modulate patterns of muscle activity which are characteristic of rapid voluntary movements. The electromyographic (EMG) activity associated with these types of movements has a distinctive "triphasic" pattern: an initial burst in the agonist is followed by a burst of antagonist activity which in turn is followed by a second burst in the agonist. A question of some interest has been the extent to which each part of this muscle activity pattern is generated by central commands or by peripheral feedback occurring during movement. We are investigating this question by determining whether MI neurons display triphasic patterns of activity during rapid voluntary movements and, if so, whether this activity precedes the bursts of muscle activity. The answer to these questions are of significant theoretical importance for the role of sensory feedback to somatic sensorimotor cortex.

The previous experiments in this project addressed these problems by examining neuronal activity in those SI areas which are known to be densely and reciprocally connected to MI. It was found that the activity of many neurons in posterior SI regions showed a striking similarity to MI neuronal activity in terms of their relation to limb position and exerted force. Such a similarity is consistent with the hypothesis that peripheral inputs to MI are relayed through SI. These findings are also consistent with the possibility that some SI neurons receive inputs from MI and are involved in the initiation of movement. We are now attempting to determine whether sensory input to MI and SI cortex reflect the magnitude of the difference between an intended movement and the movement which the limb actually executes after interaction with the environment. The other investigator on this project is Steven P. Wise, Research Biologist, Laboratory of Neurophysiology, NIMH.

Methods:

Two monkeys were seated in a chair with their arms coupled by a plastic sleeve to a servo-controlled torque motor. One monkey was trained to pronate or supinate its forearm while the other monkey was trained to flex or extend its wrist. In experiment #1, alternating active pronation or supination movements of 20° or isometric contractions were made. The movements were made with or against a steady load which was applied by the motor and were followed by periods of steady maintenance of limb position. Sensory stimuli consisting of ramp displacements of the limb (10°) were delivered during the period between movements.

In experiment #2, the monkey was conditioned to make wrist movements of two different amplitudes in each of two opposite directions. During some of the trials, the movement of the limb was halted either shortly after the beginning or before the end of these voluntary movements. This experiment allows the comparison of unit activity during control and perturbed volitional movements.

In experiment #1, unit activity and behavioral data were recorded on magnetic tape and analyzed off-line with a PDP-12 computer. For each unit recorded in experiment #1, analysis focused on the frequency and pattern of discharge at each maintained position and isometrically generated force.

In experiment #2, unit activity and event marker codes were recorded on-line with a PDP-11/03 computer. For this experiment, now in progress, it has been necessary to develop additional analytical capabilities in the off-line neurophysiological analysis prpgra, of the LNP. Accordingly, William Sheriff, of the Research Services Branch, technical development staff, has modified the off-line program to allow assessment of unit activity following the stoppage of movement of different velocities.

Experimental Findings:

Comparison of SI and MI Neuronal Activity

Analysis of single unit activity recorded for 2611 neurons in two monkeys has, thus far, produced the following similarities and differences between SI and MI.

1. Both SI and MI neurons were organized into zones with predominantly cutaneous or non-cutaneous inputs. The cutaneous zone in MI (MI/c) was located in the bank of the central sulcus, while the cutaneous zone in SI included areas 3b and 1. The non-cutaneous zone in MI was rostral to MI/c (this zone was termed MI/r) and in SI the non-cutaneous zone consisted of areas 3a, and 2.
2. The cutaneous neurons in MI and SI were similar to each other in their lack of sensitivity to maintained force or position or to the direction of active and passive movements. In addition, MI and SI neurons with non-cutaneous inputs were similar to each other, but different from cutaneous units in their sensitivity to maintained force and position and to the direction of active and passive movements.
3. A difference between MI and SI was the presence in SI of neurons with a non-muscle-like relation to force and position: a muscle which is more active when the forearm is supinated is always more active when supinating force is applied to an immovable object. In SI, a substantial proportion of cells are active with supinating force (without movement) became more active with the forearm pronated. The presence of both muscle- and non-muscle-like neurons in SI is consistent with that regions' postulated role in sensory processing. In order to interpret inputs which signal a mixture of position and force information it is necessary to have two inputs which code position and force in different ways. In that way two independent functions of two variables are created. In contrast, the lack of non-muscle-like neurons in MI is consistent with its role in motor control. MI output does not uniquely specify force or position independently. Instead it appears to specify muscle tension, which depends on both force and position.
4. In contrast to their similarity during maintained force and position, many of the non-cutaneous SI and MI neurons showed very different patterns of activity during transitions from one position or force level to another. The activity of most MI neurons resembled the pattern of EMG activity during changes in force and position. For example, a unit with increased activity related to maintained supination would almost always be characterized by a phasic increase in activity during a supination movement. In contrast, some SI neurons showed the opposite pattern, i.e. phasic decreases in activity during movements towards positions associated with tonic activity increases.

5. The activity of some MI and SI neurons showed a striking similarity to the triphasic bursting pattern of the prime-mover muscles. For example, neurons related to flexion movements displayed two distinct bursts of activity during flexion. The first burst was at the beginning of movement while the second occurred near the end of movement. During extension movements these same neurons showed a decrease or no change in activity at the beginning of movement and then increased their activity in a manner similar to antagonist muscles.

6. A quantitative comparison of the timing of each neuronal and muscle burst component is still in progress but, thus far, it is clear that the activity of some MI neurons precedes the earliest first agonist and antagonist muscle bursts by 20-50 msec. In contrast, most SI activity appears to be simultaneous with muscle activity or to follow it.

Effects of Stopping Voluntary Movement

When a movement was stopped a sustained increase in force developed against the handle and continued throughout the duration of the stop. The magnitude of this force build-up was greater when large movements were stopped than when smaller movements were stopped. Analysis of MI and SI neuronal activity has, thus far, revealed the following findings concerning the extent to which cortical neurons contribute to compensatory increases in force build-up during stopped movements.

1. Most SI and MI units displayed stop related activity that resembled the pattern of activity seen in the prime mover muscles during stops. For example, units and muscles related to flexion movements showed increased activity when flexion movements were stopped and no change or decreased activity when extension movements were stopped. Some SI and MI neurons, however, displayed stop-related activity not seen in any muscle. These neurons showed a decrease in activity when movements to which they were related (the preferred direction of movement) were stopped. MI and SI units of this type differed in their behavior when movements in the direction in which they normally showed decreased activity (the non-preferred direction of movement) were stopped. MI units of this type showed little change in activity upon stopping movements in the non-preferred direction whereas SI units showed dramatic activity increases.

2. Many MI and SI neurons showed changes in activity during stopped movements that paralleled changes in force build-up. Thus, for neurons that increased activity during flexion and decreased activity during extension there was a greater increase in activity during stops of large flexion movements than for stops of small flexion movements.

Significance to Biomedical Research and the Program of the Institute:

Further clarification of how sensory information from the periphery is utilized to initiate and control skilled motor activity is essential to a better understanding of normal and abnormal movements in man. Though much is known about the involvement of motor cortex in control of movements, more information is needed concerning the characteristics of sensory input to MI during normal and perturbed volitional movements.

Proposed Course of Project:

Analysis of the differences between cortical areas in the response of their neurons to sensory stimuli and their activity during active movements will continue. We will continue our examination of the hypothesis, first proposed by Phillips in 1969, that neurons in motor cortex should receive a neural signal that reflects the error between an intended movement and the movement that actually occurs.

Publications:

Jennings, V.A., Lamour, Y., Solis, H., and Fromm, C.: Somatosensory cortex activity related to position and force. J. Neurophysiol. 49: 1216-1229, 1983.

Evarts, E.V., Fromm, C., Krölller, J., and Jennings, V.A.: Motor cortex control of finely graded forces. J. Neurophysiol. 49: 1199-1215, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01094-03 LNP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Information Processing in the Motor Cortex		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) John P. Donoghue Staff Fellow Laboratory of Neurophysiology, NIMH		
COOPERATING UNITS (if any)		
LAB/BRANCH Laboratory of Neurophysiology		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Bethesda, Maryland 20205		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER:
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purpose of this project is to examine the <u>input-output organization</u> of neurons in the <u>motor cortex</u>. Rats have been chosen as the primary experimental animal since they are readily available and have small, lissencephalic brains. Neuroanatomical and neurophysiological techniques are being employed to characterize the first motor cortex (MI) and other closely other related cortical areas in rats and determine their connectional relationships with other neural structures. Immunocytochemical techniques are being used to identify the transmitters of cortical neurons. Neuronal activity will be monitored for several classes of <u>projection neurons</u> in the forelimb area of MI cortex in awake, behaving rats. <u>Intrinsic cortical circuits</u> are being studied by <u>intracellular injection</u> of a tracer, horseradish peroxidase, to visualize the intracortical distribution of neuronal processes of single neurons. </p>		

Objectives:

The output of motor cortex neurons is closely linked to motor activity in mammals. These cortical outputs arise after intracortical processing of inputs through a complex array of neurons. The experiments described here are designed to identify the input-output transformations that occur in the first motor (MI) subdivision of somatic sensorimotor cortex.

Our objectives are to identify each source of input to MI and the axonal targets of MI neurons by employing neuroanatomical pathway tracing techniques, to identify the transmitters used by some intrinsic and cortical projection neurons, to examine the activity of neurons in MI during motor behavior (a simple forelimb motor task), and to study aspects of the synaptic circuitry within MI. The overall objective is to gain a better understanding of routes of information flow within small modules of neocortex. The other investigators are Von Jennings, Staff Fellow, Laboratory of Neurophysiology, NIMH and Steven P. Wise, Research Biologist, Laboratory of Neurophysiology, NIMH.

Methods:

1. Identification of inputs and outputs of MI cortex. For pathway tracing experiments, axonal tracers (histochemical markers or radioactive amino acids) are injected into neurophysiologically characterized cortical regions. Following appropriate survival times the animals are perfused and the brain processed by standard methods to reveal the distribution of tracer substances in the brain and thereby the connections of the injected cortical regions.
2. Identification of cortical transmitters. The transmitters of cortical neurons are identified with immunocytochemical techniques. Antibodies to synthetic enzymes of putative neurotransmitters are applied to tissue sections from rats and the distribution of labeled neurons demonstrated by standard immunocytochemical techniques.
3. Chronic single-unit recording. Rats are trained to press a bar with their forelimb in a stereotyped manner in order to obtain a water reward. After learning the motor task, a recording chamber is placed over the forelimb region of MI cortex and stimulating electrodes are placed: (a) in the locus coeruleus or mid-brain raphe nuclei to stimulate these cortical afferent pathways or (b) in the corticospinal tract, basilar pontine nuclei, thalamus, or the contralateral cortex, in order to test for antidromic activation of the MI neurons that project to these structures. Subsequently, single unit recordings are made during task performance. Unit activity, force, and occurrence of bar pressing are recorded on-line with a PDP 11/03 computer and these data are analyzed off-line with a PDP 11/34 computer.
4. Intracortical circuitry. The intracortical connections of neurons in MI cortex are identified by intracellular recording and injection of the tracer, horseradish peroxidase (HRP). Intracellular recordings are made with glass electrodes filled with 4% HRP in tris/KCl buffer. Certain distant axonal connections of each neuron may be determined with antidromic activation methods. In some cases cortical projection neurons (such as pyramidal tract neurons) or cortical afferents (such as thalamic fibers) are labeled with anatomical tracers in the before

intracellular injection so that synaptic relations between two identified elements may be examined.

Experimental Findings.

1. Cortical field definition and pathway tracing experiments. With intracortical microstimulation and axonal transport methods, we have shown that the MI cortex of the rat coincides with a distinct cytoarchitectonic area, the lateral agranular field (AG_1), and also includes part of the adjacent granular cortex of the first somatic sensory area. We have now examined the inputs to AG_1 with axonal transport methods. We have found that AG_1 receives input from cortical somatic sensory areas and subcortical regions involved in motor control. This combination of inputs suggests that AG_1 is an important cortical region for sensorimotor integration and movement control. There are further inputs from the basal forebrain region, the midbrain raphe nuclei and the locus coeruleus. These systems are likely to have a modulatory effect on cortical output.

The outputs of motor cortex are currently being examined. We have found that MI in the rat has dense connections to structures related to movement control, such as the ventral horn of the spinal cord, the pons, and the striatum. MI also projects back to cortical and thalamic centers that provide its main input. This suggests that MI may control the types of ascending information it receives.

2. Identification of cortical transmitters. We have found that a large number of pyramidal neurons in cortical layers V and VI label with an antibody to glutaminase, an enzyme important in the synthesis of glutamate. This finding suggests that these neurons may use glutamate as a neurotransmitter. We predict that many cortical neurons that project to the spinal cord, brainstem, and thalamus use glutamate as a neurotransmitter. Fewer cells in the superficial layers label with this antibody, suggesting that many corticocortical cells could use a different, presently unidentified transmitter.

3. Chronic recording experiments. We have established a reliable method for single-unit recording in behaving rats. About 100 units have been recorded in somatic sensorimotor cortex during the forelimb task described above. We have observed many units related to force production, and they may begin this activity 100 ms prior to the generation of active force by the rat. These findings suggest that neurons in the rat MI cortex are important in the elaboration and execution of central motor programs. To identify the role of modulatory inputs on cortical discharge the locus coeruleus was stimulated during the bar pressing task. Preliminary data suggest that the locus coeruleus input enhances the activity-related discharge of some single units in AG_1 during movement, but has a direct inhibitory effect on others.

4. Intracortical circuitry. Four pyramidal tract neurons and two commissural neurons have been labeled by intracellular injection of HRP. Preliminary examination of the axonal arborizations of these cells reveals that both types of MI projection neurons have extensive intracortical connections, suggesting that they have an important role in information processing within MI as well as in sending signals to their projection targets.

Significance to Biomedical Research and to the Program of the Institute.

Elucidation of the mechanisms of cortical information processing in the motor cortex, especially the role of the different afferent inputs and cell types in providing the cortical output, will provide a better basis for understanding normal and abnormal motor function in all mammals, including humans.

Proposed Course of the Project:

Much of the anatomical work has been completed and a final report concerning the inputs to motor cortex is in press in the Journal of Comparative Neurology. Work on the transmitters of corticofugal neurons is largely complete and a manuscript is in preparation. The majority of effort is now being devoted to chronic single-unit recording from awake behaving rats and intracellular injections of identified projection neurons.

Publications:

Donoghue, J.P., and Wise, S.P.: The motor cortex of the rat: cytoarchitecture and microstimulation mapping. J. Comp. Neurol. 212: 76-88. 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01335-13 SMRA

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of Schizophrenia

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Richard J. Wyatt, M.D., Chief, Adult Psychiatry Branch, NIMH

COOPERATING UNITS (if any)

Laboratory of Preclinical Pharmacology,
Laboratory of Clinical Neuropharmacology and Harvard University

LAB/BRANCH

Adult Psychiatry Branch

SECTION

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

28

PROFESSIONAL:

27

OTHER:

1

CHECK APPROPRIATE BOX(ES)

- ☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Studies performed within the Unit on Schizophrenia include studies employing computerized axial tomography (CT) and the BEAM technique. Post mortem studies examined brain morphology and neurochemistry. Of the psychopharmacology studies, investigations have been into the disorder of tardive dyskinesia, thioridazine, reassessment of the anhedonia hypothesis, neuroleptic-induced seizures, naloxone and clonazepam. Pharmacogenetic studies have been performed to begin separating the relative influences of genetic and nongenetic variables on drug response. Biochemical studies have examined p-chlorophenylalanine, 5-HT and the indoleamine hypothesis. Studies have been performed, also, on phenylacetic acid (PAA) and phenylethylamine (PEA), water regulation and zinc. Work continues on monoamine oxidase, alpha-adrenergic receptors and calcitonin. Finally, investigations into the relationship of blinking to the schizophrenic syndrome continue as does research into the viral hypothesis of schizophrenia. Research is now underway, also, examining multiple personality disorder.

Project Description

Drs. Llewellyn B. Bigelow, Daniel R. Weinberger, Joel E. Kleinman, Craig N. Karson, Steven G. Pokin, John M. Morigisa, Dilip V. Jeste, William J. Freed, Ronald Zec, Richard L. Wagner, Bruce H. Phelps, Farouk Karoum, Lynn DeLisi, David Shore, Janice R. Stevens, Frank Putnam, H.E. Cannon-Spoor, Esa Korpi, A. Duchemin, Karen Berman, Allen Church, Grant Ko, Charles Kaufmann, William Lawson, Richard Jed Wyatt and Mr. A. Paul Oliver, Erminio Costa and Hsiu-Yang from Laboratory of Preclinical Pharmacology, Ingeborg Hanbauer from Laboratory of Clinical Neuropharmacology and Frank Duffy from Harvard University performed studies in schizophrenia.

Objectives

The ultimate goal of our research into the schizophrenic syndrome is to improve diagnostic abilities, to delineate etiologies and to develop optimum treatment methods. While attainment of this three-pronged goal remains well beyond today's grasp, our principle objective is to work towards this goal by performing basic and clinical research in a broad range of disciplines and psychiatric subspecialties. While our emphasis is in the direction of biological psychiatry, our research attempts to produce new knowledge, simultaneously synthesizing prior information in a multidisciplinary manner. The research of the Adult Psychiatry Branch pursues understanding into the schizophrenic syndrome through investigation of the psychological, biochemical, and anatomic aspects of this disease process.

Clinical Services

The clinical services for the research inpatients of the Adult Psychiatry Branch are provided under the overall supervision of the Clinical Director, Llewellyn B. Bigelow, M.D. Staff support comes from the William A. White Division of Saint Elizabeths Hospital, also under Dr. Bigelow's direction. There are three nursing units or wards, each with a capacity of 12 to 16 patients. The principal focus of the research in this branch is an effort to understand the biological causes of and to develop superior treatments for the major public health problem posed by chronic schizophrenia. It is the purpose of the clinical support services to provide first rate patient therapeutic care in a setting which also permits ethical and well-designed research. The patients, all of whom must be voluntary, are recruited from many sources. Some refer themselves, in other cases families contact the National Institutes of Health seeking assistance, and patients are often referred by several local hospitals who are aware of the treatment and research opportunities offered by our program.

Patients stay on the research units for up to two years while they participate in both research and rehabilitative programs. A full range of the latter is offered, led by the physician ward administrator and strongly supported by a highly professional nursing staff and full time social workers, occupational and recreational therapists; ancillary programs are also available. Of particular note is the work experience program known as Industrial Therapy which permits patients to work at their own rate and at tasks suited to their clinical condition. No patient is charged for services received while enrolled as a research volunteer.

A typical patient stay in the William A. White Division would be something as follows: A four to six week period is planned for initial diagnostic studies and stabilization of the patient to the new environment. Therapeutic relationships are established and a detailed

history is collected. Upon admission the patient is placed on coded medication only, usually similar to that which he was taking prior to entry into the program. This is to ensure that subsequent changes in medication may be performed "double blind" to minimize the effects of rater and patient bias in the measurement of treatment responses.

After this initial six week period has past, the patient will be switched blindly by coded medication to placebo medication. This period, lasting up to six weeks, is particularly important in that it permits the establishment of a diagnosis with the patient in a neuroleptic free state. This period also permits taking biological measurements such as spinal fluid, blood, and urine as well as performing other noninvasive procedures without contamination of results by high levels of strong medication. This drug free period is terminated if, in the opinion of the ward administrator and clinical director, such termination is wise and in the best interest of the patient or the program.

After completing the drug free interval, the patient is then placed on a fixed dose haloperidol regimen in order to establish behavioral and biological response in comparison to other patients. At the conclusion of six weeks of fixed dose haloperidol, there is a second drug free period lasting again up to six weeks during which time short term infusions are performed. These assess neuroendocrine status and provide data for other challenge studies. Assuming the patient has been shown to be somewhat responsive to neuroleptics, he is then placed again either on haloperidol or, if the response seemed less than optimal, on a different neuroleptic, usually mellaril or navane, and stabilized once again for optimal drug response. It should be noted at this time that no patient is enrolled in the program who is able, given conventional neuroleptic treatment, to lead a productive life outside the hospital setting. Therefore, all patients in our program are either nonresponsive or only insufficiently responsive to known treatment.

After the two drug free treatment periods are completed, consideration is given to assigning the patient to an appropriate protocol, involving the addition of novel medication to his neuroleptic medications currently under study. These might include lithium carbonate, fenfluramine, clonidine, and bromocriptine. All, for a variety of theoretical reasons, show some promise as at least possible candidates as therapeutic agents in the treatment of chronic schizophrenia.

A major concern of the clinical section of this Branch is to obtain valid quantitative ratings of each patient's pathology. The backbone of this system is a daily rating by nurses using a scaled version of the psychiatric rating scale, modified by Dr. Bigelow. Since no rating scale, however well conceived, can produce data any better than individuals are trained to obtain, a major effort is focused on continued fine tuning of the rating process. This is accomplished through weekly meetings of the nursing staff on both day and evening shifts to address ongoing problems.

Additional rating measures are used; such as sleep observation and physicians' ratings of negative symptoms, as circumstances dictate. The ongoing program on tardive dyskinesia also utilizes nurse movement disorder ratings weekly or more frequently. Just this year we have brought on line computer terminals into each ward that can be used for the direct entry of rating data. Maintaining data in this way has greatly increased reliability of ratings, enabling up-to-date monitoring of the ratings on each patient.

In order to oversee, monitor, and coordinate research and treatment efforts on each of the three units, the Clinical Director meets weekly with each individual ward administrator and holds weekly research rounds on each of the three units, alternating individual

management and research issues. Potential conflicts between clinical and research needs can be discussed indepth during the individual meetings with more controversial or difficult issues referred for group discussion at weekly Rounds.

The format of the weekly Rounds involves all of the research staff assembling on one of the nursing units. Each patient is interviewed by a member of the senior staff in front of the group for a brief period. Opportunity is always given for questions from the patient as well as from the research staff at large. Although at first glance such a procedure might seem intimidating for patients, experience has shown that they appreciate the interest shown and opportunity to be heard by the entire research group, most of whom are familiar to them from intermittent daily interactions. After Rounds, which last about an hour, the research group assembles for a Post-Rounds Conference where each patient on all three units is reviewed briefly and any particularly difficult problems concerning research, or clinical issues posed by a patient's condition, are discussed by the group at large.

An additional function of the Post-Rounds Conference is to distribute, as equitably as possible amongst the many investigators of the Branch, permission for participation in specific protocols. There are so many protocols and ideas under development and investigation in the Branch that no one patient can participate in all of them. Major limiting factors include but are not limited to length of time it is appropriate for a patient to be free of neuroleptic medication, the degree of difficulty of cooperation required by particular protocols, and potential biochemical complex between protocols. For instance, many protocols require a minimum two-week time elapse on stable medication in order to have neuroreceptors, theoretically at least, in a steady state. This is true particularly of challenge tests.

A current major effort is the central documentation of significant findings both of history and of current status of each patient. It is hoped within the next year to reduce this effort to a form which can be incorporated into an ongoing data base. One important aspect of this effort is the establishment of diagnoses late in the initial or second drug free period. The patient is given a structured interview. The interview is recorded on audio tape. At the conclusion of the interview, research diagnostic criteria and DSM III criteria forms are completed for each patient and a diagnosis derived. Additionally, the psychiatric rating scales and other specialized scales are completed and archived. Clinical data derived from each patient is considered the property of the entire research unit and not under the single purview of any investigator. The social worker for the Division is a skilled senior person with a special interest in family history. Family therapy is conducted regularly for those families where it is indicated if geographic obstacles can be overcome. Recreational activities are frequent with many outings to neighboring sites of interest. A recreational therapist comes in on weekends to lead activities.

In summary, the clinical activities of the Adult Psychiatry Branch have been structured to provide optimum therapeutic impact as well as a research setting for the study of the devastating syndrome of chronic schizophrenia. It is a supportive environment for investigators and patients from which it is hoped that new understanding and treatments will emerge.

Neuropsychiatry

Computerized Axial Tomography (CT)

Recent investigations with computed tomography (CT) have reopened a question raised earlier in this century by pneumoencephalography-namely, whether some chronic

schizophrenic patients have structural brain abnormalities that can be observed during life. The CT studies suggest that as a group, chronic schizophrenic patients differ from normal control subjects in the following ways: (1) They have significantly larger lateral cerebral ventricles; (2) They are more likely to have dilated cortical fissures and sulci; (3) They have a greater prevalence of apparent atrophy of the anterior cerebellar vermis; and (4) They have a greater frequency of reversed occipital lobe asymmetry, (i.e., right occipital lobe wider than left). Together, these findings indicate that CT abnormalities are suggestive of structural brain disorders, in most instances atrophy or perhaps a dysplastic process, and that they occur in some individuals diagnosed as having schizophrenia. It is unclear, however, whether these findings are relevant to the pathogenesis of a form of schizophrenia or whether they develop secondarily.

In one investigation to assess whether these findings are present at the onset of illness and the beginning of treatment, Dr. Weinberger and colleagues evaluated CT scans of 102 primarily acute psychiatric patients and 26 control subjects. The patients included 35 with first-episode schizophreniform disorder, 17 with chronic schizophrenia, 23 with affective disorders, and 27 with other psychiatric disorders. Both the schizophreniform and the chronic schizophrenic patients had significantly larger cerebral ventricles than did the other psychiatric or control subjects. Ventricular size in the affective disorder patients was not significantly different than in any of the other groups. Twenty percent of the schizophreniform patients had enlarged ventricles, i.e., outside the approximate normal limit for this age (ventricular brain ratio greater than 10). The only other subjects outside this limit were four (24%) chronic schizophrenic patients. Five schizophreniform and three affective disorder patients had evidence of mild cortical atrophy. Neither cerebellar atrophy nor reversals of expected frontal and occipital lobe asymmetries were found with increased frequency in the schizophreniform patients.

The main finding from this study of CT scans of primarily acute psychiatric patients is that seven of 35 patients (20%) with first episode schizophreniform disorders had cerebral ventricles larger than expected for the second and third decades of life. This implies that a subtle structural brain abnormality is associated not just with chronic schizophrenia but with at least some cases of schizophreniform psychosis. To conclude that the seven schizophreniform patients with larger ventricles have a structural brain abnormality requires a valid definition of the limit of normal ventricular size. Although it is unlikely that such a limit can be sharply defined, the results of CT studies of healthy individuals of this age are remarkably consistent and indicate that a ventricular brain ratio greater than 10 is rare (probably less than 5% of cases). Slight differences from one study to another in the frequency of a ventricular brain ratio greater than 10 probably reflect minor methodological idiosyncracies involved in tracing the ventricular edge. In our research, only one of the 156 non-schizophrenic subjects derived from the present and previous studies has exceeded this limit for ventricular size. The data, therefore, support the notion that a ventricular brain ratio greater than 10 is suggestive of a central nervous system abnormality.

Previous reports of enlarged ventricles in schizophrenia have involved primarily chronic, poor prognosis patients. Since many of the chronic patients had been ill for years, the possibility could not be excluded that ventricular enlargement had developed during the course of the illness and was, therefore, an epiphenomenon. In light of the present study, however, this possibility appears doubtful. Although the relationship of large ventricles to the pathogenesis of a schizophreniform disorder is unknown, it is likely that the finding predates the acute illness, probably by a period of years. The unlikely alternative, that of rapidly enlarging ventricles in an adult, typically would be accompanied by clear neurological symptoms and signs of increased intracranial pressure. The data from most other

studies support the notion that ventricular enlargement precedes the development of the illness in adult life. This supporting evidence includes the lack of correlation between length of illness and ventricular size and an association of poor premorbid adjustment with large ventricles.

The data, however, are not entirely consistent. Three studies found that ventricular enlargement was more common in older patients who had been ill longer. While this probably reflects the greater likelihood of finding poor outcome among older patients who are still hospitalized, it leaves open the possibility that ventricular enlargement in chronic schizophrenic patients comes in several forms. One variety pre-dates the illness while another may develop during the course of illness. The etiological possibilities for the latter include a degenerative brain disorder related to the progression of the illness, or to the effect of age and treatments such as drugs and institutionalization. The etiology of the former could include anything from a long list of early developmental or childhood brain insults, perhaps serving as a non-specific risk factor for the later development of a psychiatric disorder. Of course, a specific degenerative or dysplastic process that manifests clinically as schizophrenia in early adulthood is also possible. Whatever the etiology or etiologies of this former variety, it is relatively certain that neuroleptic drugs, ECT, and/or institutionalization are not responsible.

None of the 50 patients with other psychiatric disorders had a ventricular brain ratio in excess of 10. It is further interesting to note that the mean ventricular brain ratio and the standard deviation for these patients is almost identical to that reported in another study of patients with personality disorders and also to that of normal controls. The affective disorder group, though not significantly different from either controls or the "other disorders" patients, had two patients with ventricular brain ratio values greater than two standard deviations of the mean of our previously described normal control sample. This is perhaps consistent with reports that some patients with affective disorders have enlarged ventricles. In the present study, however, it is compatible with random sampling from a normal distribution. Whether affective disorder patients have enlarged ventricles or not seems less critical than whether ventricular enlargement has pathogenic and/or clinical relevance. Clearly, ventricular enlargement, especially of the magnitude found in schizophrenic patients, is a non-specific sign that can result from many causes.

In conclusion, this study strongly suggests that some schizophreniform patients have enlarged ventricles and possibly mild cortical atrophy prior to the onset of their illness and that these findings are not the result of psychiatric treatment. The larger question, whether they have pathogenic significance, is still unanswered. These patients are part of a long-term prospective project, which should help resolve whether finding ventricular enlargement or cortical atrophy at the onset of the illness has prognostic value.

Cerebral Ventricular Size and the Dopamine Hypothesis

The question arises as to the significance of cerebral ventricular size to neurochemical hypotheses in the schizophrenic syndrome. Is the factor of cerebral ventricular size relevant in testing the dopamine hypothesis of schizophrenia? The finding that neuroleptics are more effective in schizophrenic patients with normal ventricles opposed to large ventricles suggests that neurochemical differences may also exist between the two groups. Since neuroleptics are dopamine blockers this raises the possibility that schizophrenic patients with normal ventricles differ from those with large ventricles in terms of dopaminergic activity. To test this hypothesis, three dopaminergic parameters have been

examined in our patients. These include plasma prolactin concentrations, eye blink rates and cerebrospinal fluid homovanillic acid concentrations.

Dr. Kleinman and colleagues measured drug-free plasma prolactin concentrations using radioimmunoassays in 15 chronic schizophrenic patients. Since tuberoinfundibular dopamine inhibits the release of prolactin from the anterior pituitary, one would expect that increased dopaminergic activity would be reflected in low plasma prolactin concentrations. Insofar as increased dopaminergic activity is associated with increased psychopathology, there should be an inverse relationship between the latter and plasma prolactin concentrations. This was demonstrated in the 17 patients studied thus far using the Brief Psychiatric Rating Scale (BPRS) as a measure of psychopathology. Both total score and the psychosis syndrome correlated inversely with plasma prolactin concentrations. When patients were further subdivided by cerebral ventricular size as determined by CT scans, this correlation was significant for those patients with normal VBR, but not those with large VBR.

A second possible measure of dopaminergic activity is eye blinking. Dr. Karson and colleagues have developed several lines of evidence to support the notion that eye blinking reflects Central Nervous System (CNS) dopaminergic activity. These include the following: (1) Patients with decreased dopaminergic activity (Parkinson's disease) have decreased blink rates; (2) Apomorphine, a dopamine agonist increases blink rates in monkeys; (3) Pretreatment with haloperidol shifts the dose response to monkeys more sensitive to apomorphine; and (4) Dopamine blockers (neuroleptics) decrease blink rates in monkeys. With this in mind, Dr. Karson predicted that drug-free chronic schizophrenic patients should have increased blink rates if they had increased dopaminergic activity. In 56 drug-free schizophrenic patients, blink rates were increased relative to 81 controls.

Dr. Kleinman and colleagues proceeded to examine eye blinking with respect to cerebral ventricular size. No differences were found between normal and large VBR subjects in terms of drug-free blink rates. Response to neuroleptics, however, was found to be different between the two groups. Large VBR schizophrenic patients' (n=13) blink rates did not decrease with neuroleptics, while normal VBR patients (n=43) had a significant decrease in eye blink rates on neuroleptics. A third measure of dopaminergic activity is cerebrospinal fluid homovanillic acid which we have measured in drug-free schizophrenic patients. Analysis of this data is in progress.

To conclude, three studies (response to neuroleptic, relationship between psychopathology and prolactin and the effect of neuroleptics on eye blinking) support the notion that dopaminergic activity differs between patients with normal and large ventricles. It is unclear, however, whether the differences between the two groups of patients are related to increased dopaminergic activity in the normal VBR group or decreased dopaminergic activity in the large VBR group. Obviously, other strategies for testing these hypotheses are needed. More data on these studies will be presented in the post mortem studies section of this report.

Cerebral Ventricular Size and 5-HIAA

Approaching the question of whether or not enlarged cerebral ventricles has clinical significance from a slightly different perspective, Dr. Potkin and colleagues examined serotonin's major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in cerebrospinal fluid (CSF) of schizophrenic patients. The hypothesis that serotonin may be involved in the pathogenesis of at least some forms of schizophrenia is one of the oldest neurochemical theories of this disorder. By subgrouping patients on the basis of CSF 5-HIAA and grouping

these patients, also, according to cerebral ventricular size, it was hoped that new insights into both neurochemical and neuroanatomical aspects of schizophrenia might accrue.

Among the 24 schizophrenic subjects and 16 neurological controls, no differences in CSF 5-HIAA concentrations were found. There were no differences in CSF 5-HIAA between the medication-free schizophrenic patients and the schizophrenic patients on neuroleptics. The nine schizophrenic patients with abnormal cerebral ventricles had much lower CSF 5-HIAA than the 15 with normal ventricles. While three of the normal ventricle schizophrenics had higher 5-HIAA than any of the other subjects, we could find no distinguishing clinical characteristics of these three patients. Similarly, two of the abnormal ventricle schizophrenics had lower 5-HIAA than any of the other subjects. They, as well, were clinically indistinguishable from the other schizophrenic patients.

That we found no difference in CSF 5-HIAA between our total schizophrenic group and the controls is consistent with other researchers' findings. That we found decreased CSF 5-HIAA in the subgroup of schizophrenics with enlarged ventricles supports our suspicion that this morphological abnormality may be a differentiating factor in subtyping various schizophrenic etiologies.

CT Data, Plasma Prolactin and the Dopamine Hypothesis

Approaching the dopamine hypothesis from yet another perspective, Dr. Kleinman and colleagues examined plasma prolactin concentrations in 17 drug-free chronic schizophrenics. Serum prolactin concentration is one peripheral measure of central nervous system (CNS) dopaminergic activity. It is an anterior pituitary hormone whose release is tonically inhibited by hypothalamic dopaminergic neurons. It has been hypothesized further that if schizophrenic patients have increased dopaminergic activity, they also might have decreased serum prolactin concentrations. Although investigators have consistently failed to verify the decreased serum prolactin hypothesis, psychopathology in drug-free chronic schizophrenic patients has been shown to correlate inversely with serum prolactin concentration. This finding suggests a relationship between the degree of dopaminergic activity and psychotic symptomatology at least in this group of patients.

These hypotheses were integrating with the recent computed tomography (CT) data demonstrating that some schizophrenic patients have large cerebral ventricles. Patients with large cerebral ventricles have been hypothesized, also, to have a defect state or negative symptoms (flat affect and poverty of speech) that are unrelated to dopaminergic activity. Insofar as patients with normal ventricular size are better responders to dopamine-blocking drugs, one might expect that psychopathology in these subjects may be related to dopaminergic activity as reflected by plasma prolactin concentrations. This should be particularly clear with regard to positive psychiatric symptoms (hallucinations, delusions and thought disorders) that are responsive to dopamine blockers. This study tested this hypothesis in drug-free chronic schizophrenic patients divided into two groups on the basis of cerebral ventricular size.

In the 17 chronic schizophrenic patients studied, median plasma prolactin concentrations varied inversely with total Brief Psychiatric Rating Scale (BPRS) scores for drug-free chronic schizophrenic patients. This relationship is particularly clear for the patients with normal ventricular size and is less clear for patients with larger ventricular size. While the significance of this finding is not yet fully understood, these results do show a relationship between plasma prolactin concentration and psychiatric symptoms in chronic schizophrenic patients. Also, these results are consistent with previously reported results.

BEAM

As we explained in last year's report it is generally accepted that schizophrenic patients as a group have a greater occurrence of EEG abnormalities than do normal individuals. There is, however, no consensus as to which, if any, abnormalities predominate or are important. Contributing to this problem has been the difficulty in interpreting the massive quantities of data that are generated by multiple electrode recordings from the human scalp, although numerous ingenious approaches have been suggested.

Recently, advances in solid state technology have produced a technique of brain electrical activity mapping (BEAM) that creates color maps of condensed and summarized EEG or evoked potential data and displays them on an ordinary color television. The technique has been useful in investigating neurologic problems ranging from an anatomically well defined lesion or brain tumor, to a functional deficit or dyslexia. Our work introduces this method to schizophrenia research and demonstrates the applicability of the BEAM technique to the search for biological markers in this disorder.

In the past year, Dr. Morihisa has continued to perform research in electroencephalography and evoked potentials in normal and schizophrenic subjects. The data are now being analyzed. Also, Dr. Morihisa, in collaboration with Drs. Kleinman and Weinberger, has established a neurophysiology lab that utilizes Brain Electrical Activity Mapping technique.

Post Mortem Studies

In 1972, Drs. Rosenthal and Bigelow reported on a post mortem quantitative study of 10 brains from schizophrenic and 10 brains from control patients. The only significant difference among the 10 measures taken was the presence of an increased mean thickness of the corpus callosum in the schizophrenics. Although this study has been cited fairly frequently, no replication study has appeared in the literature. In view of the intense recent interest in interhemispheric communications, mediated largely by the corpus callosum, Drs. Bigelow, Nasrallah and Rauscher performed a follow-up study on the thickness of the corpus callosum in autopsied brains of another group of schizophrenic and control subjects.

The sample consisted of all brains removed and adequately preserved in formalin at Saint Elizabeths Hospital during the years 1972 through 1977 unless the pathology report indicated the presence of gross brain damage such as severe atrophy, hemorrhage or infarction. The 64 brains available for study had been previously sectioned coronally. Two of the investigators, blind to diagnosis and history of the subject, measured the thickness of the corpus callosum. The overall mean thickness of the callosum was calculated from five to eight bilateral measures. Additionally, a mean sectional width was calculated from the mean of the two most anterior, two middle and two most posterior section faces available. Most brains had four sections suitable for measurement, but occasionally only three slices were present. Another of the investigators reviewed the chart of each patient to establish diagnosis. Eight subjects could not be diagnosed because of inadequate information or the presence of evidence for two or more major diagnoses such as alcoholism and schizophrenia.

Results showed the death-autopsy interval to be significantly greater in the early onset vs the late onset group ($p=0.04$) and the neurological group ($p=.03$). Although there is a small correlation between the width of the corpus callosum and the death-autopsy interval for the entire group (Pearson $r=0.29$; $p=0.03$), this correlation is not significant for any of the individual groups. There was no correlation between brain weight and corpus callosum

thickness ($r=0.25$; $p=NS$). The mean thickness of the corpus callosum was significantly greater in the early onset schizophrenic group than in any other group.

This difference appears to be a function of an increase in the anterior and middle portions of this structure, since significant intergroup differences were not found for the posterior. There was a significant correlation of age at death with all the measures of callosal thickness ($r=-0.48$; $p=0.001$ for the overall mean). When the results were covaried for age at death, however, the significant inter-group differences remained. Examination of this relationship revealed that it held only for the two schizophrenic groups and not for the neurological or other psychiatric diagnoses.

These results confirm in part the original report of increased thickness of the corpus callosum in chronic schizophrenia. The difference between the schizophrenic and control group is not as great in this study as in the former one, but this discrepancy could be due to the difference in populations available for study. The earlier study used control subjects diagnosed as sociopathic personality disorder and had four subjects without psychiatric or neuroleptic diagnosis. The intervening years have witnessed the outplacement from psychiatric hospitals of such individuals.

Another difference between this and the earlier sample is the increased age at death of the current group. The significant negative correlation between corpus callosum thickness and age at death may account in part for the lower mean callosal thickness reported here for the schizophrenic group as compared with the original study, (6.11 mm in the 1972 report of Rosenthal and Bigelow vs 4.96 mm in this study). The mean age at death was 56 years in the previous study and 67 years in this study. It may be meaningful that there is a significant negative correlation of callosal width with age only in the two schizophrenic groups and not in the two control groups. This finding could be consistent with a pathological process such as a sub-clinical viral infection leading initially to a slightly increased tissue volume and later resolving after cell loss, thereby leading to a relatively normal dimension.

Morphology

A second group of studies of morphology has been conducted by Dr. Weinberger and colleagues using the Yakovlev collection as a resource. One study from this collection involved the measurement of the size of the anterior vermis of the cerebellum. It was determined that the anterior vermis of the cerebellum was decreased in some schizophrenic patients relative to controls. This confirmed the previously mentioned finding of decreased vermal size in schizophrenic patients seen on CT scans.

Neurochemistry

In addition to morphological studies, the Adult Psychiatry Branch has been active in studying post mortem brains for neurochemical studies. Dr. Kleinman has collected approximately 300 brains of patients with schizophrenia, affective disorders, heroin and alcohol addiction and normals. Twenty different brain regions have been used for measurements of catecholaminergic metabolites, indoleamines and metabolites, several neuroleptics, and several receptor or binding sites. The major effort has been in testing monoamine hypotheses of mental illness.

Dr. Kleinman and colleagues have measured catecholamines and their metabolites in nucleus accumbens, hypothalamus and substantia nigra of schizophrenic patients, other

psychotic disorders and normals. No differences were found for dopamine, homovanillic acid or 3,4-dihydroxyphenylacetic acid. Increased norepinephrine and 3-methoxy-4-dihydroxyphenylglycol were found in chronic paranoid schizophrenic patients (n=10) relative to other patients (n=17) and normals (n=17) in nucleus accumbens and hypothalamus. Although a neuroleptic effect cannot be ruled out, the fact that the patients with neuroleptic treatment do not have these increases makes this finding less likely to be attributed to neuroleptic treatment alone.

Several binding sites and receptors have also been measured in schizophrenic brains and controls. Dr. Kleinman and colleagues measured six radioactive ligands in caudate nuclei from 10 chronic schizophrenic patients and 10 controls. These included ^3H -spiroperidol, ^3H WB-4101 (α -receptors), ^3H -dihydroxyphenolol (β -receptors), ^3H -diazepam, ^3H -naloxone, ^3H -QNB, and ^3H -GTP. The only significant difference was increased ^3H -spiroperidol binding (probably dopamine type two receptors) in schizophrenic caudate nuclei relative to controls. Also, Dr. Kaufmann and colleagues have measured ^3H -HQNB (muscarinic) in pons, frontal cortex and hypothalamus of suicides and controls. No significant difference has been found.

In related work performed by Drs. Kleinman, Costa and Hanbauer looking at dopamine type one receptors, dopamine-sensitive adenylate cyclase activity was not found to be different between schizophrenic patients or controls even in basal activity or after dopamine stimulation in caudate, nucleus accumbens, hippocampus or cerebellum. Stimulation of the enzyme with sodium fluoride or a GTP analogue yielded greater increases in schizophrenics caudate nuclei or nucleus accumbens, but not in hippocampus or cerebellum. Moreover, when a D_1 agonist, was used, schizophrenic caudate or n. accumbens had greater increases in adenylate cyclase activity than controls. Again, a neuroleptic effect cannot be ruled out, but a small number (n=2) of subjects on neuroleptic treatment do not show the effect.

Another group of studies of the brain has been carried out studying neuropeptides with radioimmunoassays. Dr. Kleinman and colleagues have participated in these studies which to date have involved over 100 brains and seven brain regions. The major findings at present show reduced met-enkephalin concentrations in caudate nuclei of chronic paranoid schizophrenic patients. The failure to find this reduction in chronic undifferentiated schizophrenic patients or other psychotic disorder patients on neuroleptics makes a neuroleptic effect less likely, although it does not rule it out. Interestingly enough heroin addicts had reduced met-enkephalin in the caudate nuclei. This is in contrast to the decreased met-enkephalin in the pituitary work performed by Drs. Yang, Govani and Kleinman. Another preliminary finding was increased caudate substance P in the patients with other psychoses. No changes were found for neurotensin or cholecystokinin (CCK) although a preliminary finding for CCK binding was found to be increased in the amygdala of chronic schizophrenic patients (n=12), a finding which obviously needs further replication.

Finally, serotonin and 5HIAA have been studied in about 50 brains and over a dozen brain regions. Serotonin in concentrations are decreased in the hypothalamus of suicides, while increases were found in chronic schizophrenic patients in the basal ganglia. Further work needs to be performed in future.

In short, post mortem studies appear to be a valid way to test or generate hypotheses involving mental illness. The Adult Psychiatry Branch plans to continue and expand this area of research.

Psychopharmacology

Neuroleptics and Tardive Dyskinesia

The development of the neuroleptic drugs, in the 1950's, revolutionized the treatment of psychosis. Unfortunately for many patients, however, these drugs have a very serious side effect - tardive dyskinesia. The still-unfolding story of neuroleptic-induced tardive dyskinesia has considerable relevance, not only clinically but also historically, philosophicaly, and legally for psychiatry as well as for the rest of medicine.

Neuroleptics have been repeatedly found to be the best available treatment for schizophrenia. Indeed, they are appropriately given a lion's share of credit for the dramatic drop in the number of public mental hospital patients from over 500,000 in 1955 to well under 200,000 by the late 1970's. But the history of neuroleptics and tardive dyskinesia shows how a valuable treatment modality may be overused and abused, resulting in extremist calls for abandoning use altogether. Regrettably, the proven efficacy of neuroleptics in schizophrenia was viewed by some as a green light to use these drugs in large doses, for prolonged periods and for disorders in which the value of neuroleptics remained to be established. The progressive rise in the reported prevalence of tardive dyskinesia among neuroleptic-treated patients is, at least partly, attributable to the increasing use of these agents, often in high doses. According to some estimates, there may be about 100,000 patients with tardive dyskinesia in the United States alone. Yet banishing neuroleptics from psychiatric treatment because of the risk of tardive dyskinesia, would be like throwing the baby out with the bath-water. Judicious use of these drugs for specific indications, in individualized and smallest effective doses is still required.

Because these drugs are so important and effective in the treatment of psychosis and, simultaneously, cause tardive dyskinesia in so many patients, increasing research resources are being devoted to its study. Research into tardive dyskinesia in the Adult Psychiatry Branch began in 1977 and continues to expand in various directions. Our tardive dyskinesia work, spearheaded by Dr. Dilip Jeste and executed with a team approach of collaborating physicians from this Branch and other laboratories, studies the pharmacokinetic and psychopharmacological mechanisms of this disorder.

In one study, clinical signs of tardive dyskinesia as well as plasma dopamine- β -hydroxylase and platelet monoamine oxidase activities were investigated in 12 female inpatients, and 13 without persistent tardive dyskinesia. These biochemical measures remained stable over time in spite of medication changes. Tardive dyskinesia was associated with higher plasma dopamine- β -hydroxylase and lower monoamine oxidase activities both initially and at follow-up. In two patients, an apparent elevation in dopamine- β -hydroxylase activity preceded the onset of clinical dyskinesia, suggesting that elevated plasma dopamine- β -hydroxylase activity might be a potential risk marker for the development of tardive dyskinesia, at least in some patients.

Pursuing the dopamine- β -hydroxylase relationship to tardive dyskinesia further, Dr. Jeste and colleagues hypothesized that tardive dyskinesia patients with high plasma dopamine- β -hydroxylase activity would also have other enzymatic alterations suggestive of noradrenergic hyperactivity. Plasma renin activity was chosen as a peripheral indicator of noradrenergic function and plasma renin activity was measured in three groups of female psychiatric patients over the age of 50. The three groups were composed of a tardive dyskinesia group with high plasma dopamine- β -hydroxylase activity, a tardive dyskinesia with low plasma dopamine- β -hydroxylase group.

The results showed that the high dopamine- β -hydroxylase tardive dyskinesia group had significantly greater plasma renin activity than the other two groups. Our results are consistent with the hypothesis that a subgroup of tardive dyskinesia patients has relative noradrenergic hyperactivity.

To frame these results within a larger context, the most popular hypothesis of the pathophysiology of tardive dyskinesia attributes the disorder to central postsynaptic dopamine receptor supersensitivity. Yet the available evidence indicates that dopaminergic supersensitivity is probably a normal consequence of neuroleptic treatment and that other mechanisms, such as noradrenergic hyperactivity, may be necessary for the development of tardive dyskinesia. Our findings are consistent with such a possibility, at least in a subgroup of patients with tardive dyskinesia. It should be emphasized, however, that a positive relationship between dopamine- β -hydroxylase and renin activities in the plasma and central noradrenergic function have not yet been established. Nevertheless, reports of improvement in tardive dyskinesia with a β -adrenergic blocker, propranolol, and dopamine- β -hydroxylase inhibitors such as fusaric acid and disulfiram support the hypothesis that some tardive dyskinesia patients have central noradrenergic hyperactivity. Parkinson disease is associated not only with dopaminergic but also with noradrenergic deficits and is presumed to be a "biochemical opposite" of tardive dyskinesia. Because it is conceivable that disturbances of other neurochemical systems (e.g., cholinergic, GABA-ergic) may be related to the pathophysiology of tardive dyskinesia in the low-DBH, low-PRA patients, further studies of other biochemical parameters and pharmacological manipulations in different subgroups of tardive dyskinesia patients are warranted.

Finally, since prevention, treatment and cure are the ultimate goals of all medical research, Drs. Jeste and Wyatt reviewed 285 treatment studies involving more than 3,000 patients with neuroleptic-induced tardive dyskinesia. The results show that the "state-of-the-art" regarding treatment of tardive dyskinesia is rather unsatisfactory. Nevertheless, some previously defined hypotheses were supported and several promising research avenues were delineated.

It has been hypothesized that neuroleptics mask or suppress tardive dyskinesia (while, simultaneously, being the most common cause of tardive dyskinesia) by inhibiting catecholaminergic overactivity in the nigrostriatal system. Data analyzed supported this position in that at least 50% of the patients treated with neuroleptics improved. Significant symptom suppression occurred in about two thirds of the patients studied. These drugs seem to possess a specific antidyskinetic action. Also, commonly used neuroleptics in equivalent doses have comparable dyskinesia-suppressing effects. The improvement brought about by neuroleptic administration, however, usually results in at least a temporary recurrence of dyskinesia. These data suggest that, perhaps, slow but progressive reduction in neuroleptic dosages during a period of many months to several years may be a treatment method deserving careful consideration.

Although the tardive dyskinesia work is one of the most extensive aspects of our Branch's neuroleptic research, because of the pervasiveness of neuroleptic drugs in the treatment of schizophrenia, other neuroleptic studies have been performed. One example is the investigations into serum thioridazine concentrations with liquid chromatography versus radioreceptor assay.

Thioridazine

The radioreceptor assay for neuroleptics that measures displacement of ^3H -spiroperidol from striatal membrane preparation, has been used by several groups of investigators to quantify neuroleptic activity in biological samples. The rationale for the use of the radioreceptor assay is that: 1) The potency of different neuroleptics in this assay correlates highly with their average clinical potency; 2) The radioreceptor assay by measuring the total neuroleptic activity in a biological sample quantifies all active biotransformation products together with the parent compound; 3) The same relatively simple assay can be used for the quantification of structurally different neuroleptics and their active biotransformation products. No attempts, however, have been made to compare the results of the radioreceptor assay in quantifying thioridazine to assays using other methods, such as chromatography. Therefore, Dr. Linnoila and colleagues compared a neuroleptic radioreceptor assay with a high performance liquid chromatographic assay for the quantification of thioridazine and its putatively active biotransformation products.

The main finding of this study was the widely disparate results of high performance liquid chromatographic and radioreceptor assays in the quantification of thioridazine. No systematic or proportional error explained the difference. Rather the results of the two assays varied randomly from one patient to another. These findings suggest a conclusion that although the radioreceptor assay for neuroleptics seems to function satisfactorily in quantifying butyrophenones, it produces such variable measurements of thioridazine that its application to decisions involving dose adjustments of this compound in individual patients is premature.

The Anhedonia Hypothesis Revisited

Another study examining the effects, or suspected effects of neuroleptic treatment is a reassessment of existing data by Drs. Freed and Zec with an eye to sedation as a possible explanation for the anhedonia hypothesis. The anhedonia hypothesis states that anhedonia is one of the primary actions of neuroleptics. Anhedonia is described as the blunting of the effectiveness of reinforcers, impairment of goal-directed behavior, and loss of the subjective experience of pleasure. Studies supporting the anhedonia hypothesis involve acute administration of neuroleptic drugs. The therapeutic response to neuroleptics, however, develops gradually over the course of several weeks or months. Acute administration of neuroleptics primarily produces sedation, which tends to disappear with chronic use. Therefore, acute studies of neuroleptics in animals may be of limited relevance to the actions of neuroleptics in humans, particularly in the treatment of schizophrenia.

After examining the data, it was found that the presence of anhedonia was inferred from decreases in behavioral output in animals tested under direct influence of the drug. Other results presented are consistent, also, with a sedation interpretation. Several crucial experiments cited to support the anhedonia hypothesis were found to be more parsimoniously explained in terms of diffuse sedation, and no experiment conclusively ruled out this interpretation. Finally, that the anhedonia hypothesis results from animal studies makes its applicability to humans questionable. Therefore, Drs. Freed's and Zec's research challenges the anhedonia hypothesis of neuroleptics and suggests much more research is needed before this hypothesis can be seriously considered or clinically useful.

Neuroleptics and Seizures

A final direction our neuroleptic research has taken over the last reporting year examined the relationship between neuroleptics and seizures. The occurrence of spontaneous seizures during neuroleptic treatment is a relatively infrequent yet well-established phenomenon. Within a year after introduction of chlorpromazine into clinical practice in 1952, a chlorpromazine-induced seizure was reported. Such seizures occur in less than 2% of all patients taking neuroleptics, but in about 10% of patients receiving more than 1,000 mg per day of chlorpromazine or an equivalent. Seizures are more frequent in patients suffering from convulsive or organic disorders and tend to occur shortly after initiation of therapy or during an increase of dosage.

While chlorpromazine has been most frequently cited as the offending agent, this finding is controversial. Also still controversial is the crucial issue of which neuroleptics might produce the least seizures. Although the strong anticholinergic action of thioridazine might be hypothesized to reduce seizures as they reduce the frequency of extrapyramidal reactions, both thioridazine and clozapine, medications with strong anticholinergic effects, have been implicated in seizures.

Aside from clinical reports, other information may be relevant in determining the epileptogenic effect of specific neuroleptics. For example, it has been reported that, in general, the more sedative neuroleptics have a greater slowing effect on the EEG than the less sedative and more potent neuroleptics. There are, however, exceptions. Further, in vivo screening for epileptic side effects of drugs may be hampered by complications such as changes in blood flow or respiratory difficulties induced by convulsant effect but not directly attributable to the drug.

These problems can be circumvented in part by use of a recently developed in vitro assay, the hippocampal slice. The slice has been used in our laboratory to screen anticonvulsant drugs. Mr. Paul Oliver and his colleagues adapted it to assay the convulsant potential of neuroleptics in an attempt to answer several questions:

- 1) What is the epileptogenic potential of a neuroleptic agent?
- 2) How do they compare?
- 3) Can we develop a rationale for clinical guidance?
- 4) Can we relate the epileptogenic potential to some known biochemical/physiological effect of each drug?

Using the hippocampal slice method with chlorpromazine, thioridazine, haloperidol, fluphenazine, pimozide, butaclamol, molindone, combinations of neuroleptics and with benzodiazepine and neuroleptic combinations, striking differences in inducing spike activity were found.

Dose-response curves showed that chlorpromazine and thioridazine increase spike activity at low doses, but are inhibitory at higher doses. Fluphenazine and haloperidol induce an excitability response that increases with increasing concentrations and reaches a plateau at concentrations greater than usual therapeutic concentrations. Pimozide gave a response qualitatively resembling that of chlorpromazine and thioridazine, but with far less maximum excitability. Butaclamol, a potent dopamine blocker, was almost devoid of an effect, with molindone producing, if anything, a decrease in spike activity. These differences cannot be easily reconciled with any single hypothesis relating receptor affinity, clinical potency, and epileptogenic potency. In particular, the potential of neuroleptics to

increase spike activity in hippocampal slices does not appear to be directly related to their dopamine-blocking action.

Our findings do not fully support current notions as to which neuroleptics will have the fewest seizure-inducing effects. They are not consistent with the view that neuroleptics with strong anticholinergic effects tend not to produce seizures. A drug recommended by proponents of this hypothesis, thioridazine, which has very strong anticholinergic effects, significantly ($p < .001$) increased spike activity. Another hypothesis is that "sedative" neuroleptics will produce more seizures than those that are less sedative. This notion finds only partial support in our results, since chlorpromazine and thioridazine, both neuroleptics with strong sedative effects, have a significant ($p < .001$) ability to induce spiking. But according to such an hypothesis, haloperidol should have little effect on spike activity, which was not the case. Finally, our results only partially support the view that the more "potent" neuroleptics might produce fewer seizures. Although there was a negative (but not statistically significant) correlation between the spiroperidol receptor affinity of a neuroleptic and its ability to induce spike activity, there were several exceptions. In particular, haloperidol, which is approximately as potent as fluphenazine and pimozide, had far greater effect on spike activity.

Finally, our results suggest that the seizure-inducing effects of neuroleptics may be inhibited by conventional anticonvulsant medications, such as the benzodiazepines. The addition of an anticonvulsant to the drug regimen of patients whose seizures cannot be controlled by changing neuroleptics would seem warranted. Our results also suggest that present hypotheses cannot be used as guidance in choosing a neuroleptic with minimal seizure-inducing effects. Instead, it will be important to test drugs individually. Drawing on our own results with the hippocampal slice model, of those neuroleptics available for routine use in the United States (pimozide and butaclamol are not), molindone appears to be the safest. This choice is tentative; testing of additional neuroleptics may suggest alternatives.

Naloxone

Another piece of research investigating differential neuroleptic action examined the effect of the opiate alkaloid naloxone. The discovery of opiate receptors in the early 1970's and endogenous opiates in 1975 has stimulated several hypotheses of their role in mental illness. One strategy for testing these hypotheses involves administering naloxone, a narcotic antagonist, to psychiatric patients. Naloxone has been reported to reduce auditory hallucinations and other psychotic symptoms in chronic schizophrenics and to reduce euphoria-grandiosity and arousal-activation syndromes in manic-depressives. Other studies have been essentially negative, although improvement has been noted on selected items such as abnormal thought content and the continuous performance test. Similarly, naloxone decreases serum prolactin concentrations in nonhuman primates while causing no changes in manic-depressive patients. Prolactin concentrations in normal males have either decreased or not changed. A trend toward increased serum growth hormone concentrations has also been observed. Concurrent psychotropic medication and different subtypes of patients may account for some of these discrepancies. In an effort to clarify further these behavioral and hormonal discrepancies, we studied the effects of naloxone on behavior and neuroendocrine functions in drug-free chronic schizophrenics and medicated chronic schizophrenics.

What Dr. Kleinman and colleagues found was that naloxone led to a statistically significant improvement in the abnormal thought content item on the Brief Psychiatric Rating Scale only among schizophrenic patients on neuroleptic drugs. This finding confirms

an earlier report of improvement on this measure. That naloxone caused improvement only in neuroleptic-treated patients, however, requires further examination. One possible mechanism for naloxone being more effective in neuroleptic-treated patients might be an increase in serum neuroleptic concentrations. This line of reasoning requires further study.

Clonazepam

This study does not pertain to neuroleptic drugs but to the benzodiazepine clonazepam. The drugs commonly known as valium and librium are of the benzodiazepine group. Dr. Craig Karson and colleagues examined the effect of the benzodiazepine clonazepam because 1) it is the most potent agonist of benzodiazepine and gamma-aminobutyric acid (GABA) receptors clinically available in the United States, 2) because it is an effective anticonvulsant and has been advocated as a treatment for tardive dyskinesia and finally, 3) because during a test of clonazepam's efficacy for chronic schizophrenic patients with tardive dyskinesia, Dr. Karson and colleagues observed a psychological improvement in a 21-year-old man. Clonazepam treatment in this subject was associated with a reduction in assaultive behavior and incoherence of speech. This observation, combined with recent reports suggesting a role for high-dose benzodiazepines, particularly diazepam, in the treatment of schizophrenia and the efficacy of lorazepam in the treatment of acute psychosis, a therapeutic trial of clonazepam with schizophrenic patients was attempted.

Of the 13 patients who began the protocol, nine patients completed the study. Clonazepam had no significant effect on the symptoms of these patients. Four patients demonstrated aggressive behavior during the study, with only one of the four having a history of aggressive behavior. In two cases the aggressive behavior occurred during clonazepam withdrawal.

We, therefore, conclude that unlike our previous, uncontrolled observation, clonazepam provided no additional therapeutic benefit to chronic schizophrenic patients already receiving neuroleptic medications. Because clonazepam is a powerful GABA agonist, these findings do not support the hypothesis that there is a GABA deficiency in chronic schizophrenia. It is possible, however, that the concomitant neuroleptic treatment masked any beneficial effects of clonazepam, so caution is necessary in interpreting the therapeutic and neurochemical implications of these data. It is worth noting, nonetheless, that the finding of aggressive behavior in our patients is consistent with the previous association of aggressive behavior in children treated with clonazepam and the reports of violent behavior linked to another benzodiazepine diazepam.

Pharmacogenetics

Pharmacogenetics deals with significant hereditary variations in response to drugs. Such a definition is a balance between a too restrictive use of the term (referring only to hereditary conditions involving adverse reactions to drugs, such a glucose-6-phosphate dehydrogenase deficiency with abnormal response to aspirin), and too broad a definition of the term (dealing with any condition in which drug response is influenced by genetic factors). Clinical studies of pharmacogenetics are difficult because of variable contributions of hereditary and environmental factors to almost every instance of responsiveness to drugs. This fact applies no less to psychotropic drugs than to other types of chemical agents. Large-scale well-controlled, prospective studies of psychopharmacogenetics are very difficult to conduct because of practical and ethical considerations, as well as the problematic interpretation of results. While animal studies are easier to carry out, the

interpretation of such data is hampered, also, by difficulties in separating the relative influences of genetic and nongenetic variables on drug response.

The development of recombinant inbred strains of animals has provided a unique approach to studying pharmacogenetics, including psychopharmacogenetics. This technique makes available, at the same time, animals from several generations: a pair of progenitor of parent strains (which are selected because of behavioral differences between the two, and each is then inbred for at least 20 generations), their reciprocal hybrids (called F-1 hybrids) and a number of recombinant inbred strains (so called because each has a variable combination of genes from the two parent strains, and each of these strains is inbred for at least 20 generations). Inbreeding is achieved by brother-sister mating and is expected to result in a progressively increasing degree of homozygosity. Inbreeding for 20 generations should produce almost 100% intra-strain homozygosity.

In his experiments, Dr. Dilip Jeste used two progenitor strains, their F-1 hybrids and seven recombinant inbred strains. He studied the behavioral effects of β -phenylethylamine and phencyclidine. β -phenylethylamine (PEA) is an endogenous amphetamine-like compound that is normally present in the brain. Two earlier studies reported a significantly higher 24-hour urinary excretion of PEA by paranoid schizophrenic patients as compared with controls. Phencyclidine (PCP) is an exogenous hallucinogen. While the principal biochemical effects of PEA are noradrenergic and dopaminergic those of PCP are mainly serotonergic and dopaminergic.

Dr. Jeste found that the PEA-responsiveness seemed to be primarily under the control of a single major autosomal gene. There was no significant correlation between responsiveness to PEA and to PCP. The differences in the pharmacogenetics of PEA and PCP may reflect differences either in their modes of action (noradrenergic vs serotonergic) or in their pharmacokinetics.

Biochemical Studies

p-Chlorophenylalanine, 5-HT and the Indoleamine Hypothesis

Abnormal indoleamine metabolism in schizophrenia was hypothesized nearly 30 years ago and yet, evidence for this hypothesis remains circumstantial. Early support came from studies of the hallucinogenic properties of LSD which alters serotonin (5HT) metabolism and from clinical success with reserpine which depletes brain 5-HT as well as other amine stores. Measurements of indoles and their metabolites in urine, blood, and cerebrospinal fluid (CSF) of schizophrenic patients, however, have yielded inconclusive results. Several studies have found decreased concentrations of the 5-HT metabolite, 5-hydroxyindoleacetic acid (SHIAA), in the CSF and urine of schizophrenic patients while others have found 5-HIAA concentrations to be altered. In addition, elevated blood 5-HT concentrations have been reported in at least a subgroup of schizophrenic patients.

To examine the possible relationship between abnormal indole metabolism and the clinical symptomatology of schizophrenia, tryptophan, and 5-hydroxytryptophan (5HTP), the immediate precursor of 5-HT, were administered in separate double-blind trials to schizophrenic patients. Since both tryptophan and 5-HTP aggravated the psychosis of some patients, we postulated that administering a drug that would lower 5-HT might decrease clinical symptoms if 5-HT were etiologically related to the disorder.

p-Chlorophenylalanine (PCPA), an inhibitor of tryptophan hydroxylase, the enzyme involved in the rate-limiting step of the synthesis of 5-HT, is known to cause substantial decreases in brain 5-HT concentrations in animals, and to decrease CSF and urine 5-HIAA concentrations in humans. PCPA has been used for the treatment of carcinoid syndrome, reducing the elevations in whole blood 5-HT and alleviating peripheral gastrointestinal and vascular symptoms associated with the illness.

Although 5-HT has long been thought to be relevant to the etiology of schizophrenia, the use of PCPA for the treatment of schizophrenia has been reported only once in the literature. Clinical improvement was noted in a small group of acutely psychotic schizophrenic patients during a brief open trial of PCPA. To further investigate this finding and our previous results with 5HTP, Dr. DeLisi and colleagues administered PCPA in a double-blind trial to seven actively symptomatic chronic schizophrenic patients.

The results showed no significant differences between placebo and active PCPA trial periods for all seven patients as a group. The nurses' ratings showed a significant reduction in the withdrawal symptom cluster and a trend towards increased activation ratings with active PCPA. These changes were not, however, confirmed by the psychiatrist's ratings.

When examined individually, one of seven patients had a significant improvement in total BPRS scores, which was particularly evident in the ratings of paranoia, anxiety, and agitation. This change was noticeable clinically, as well as by both nurses' and doctor's ratings during the active period. He was therefore given a second trial of active drug and placebo. No detectable improvement was found during this second active period.

Our results showed that p-chlorophenylalanine, at a dose of 3000 mg/24 hr, did not produce significant clinical improvement in seven chronic schizophrenic patients, contrary to a previous report of success with this agent. This apparent discrepancy may have resulted from differences in patient selection. Our patients were a subgroup of chronic treatment-resistant schizophrenic patients in contrast to the acutely psychotic patients in the positive study. While it is possible that poor response to neuroleptic treatment may be indicative of an underlying pathologic process that might generalize to other pharmacologic treatments, patients with CT scan abnormalities were also the ones who in one study had elevated whole blood 5-HT concentrations. Four of the seven patients in this present PCPA trial had significantly elevated baseline whole blood 5-HT concentrations as well as abnormal CT scans.

Although this study has far from disproven the "5-HT and indoleamine hypothesis" of schizophrenia, failure to initiate clinical change after inhibition of the rate-limiting enzyme, tryptophan hydroxylase, makes this hypothesis less plausible. Furthermore, it appears unlikely that PCPA will be of future benefit for alleviation of schizophrenic symptoms.

Phenylacetic Acid

Phenylacetic acid (PAA) is a major metabolite of phenylethylamine (PEA) in man. Because of PEA's behavioral similarities to amphetamine, it has been the focus of considerable research since the early 1960's. Recently, PEA excretion was reported to be elevated in some chronic paranoid schizophrenic patients as well as in a group of bipolar depressed patients with psychotic behavior. Because of PEA's possible importance in psychiatric disorders, future research on PEA will inevitably require consideration of its metabolism. We have previously reported a gas-chromatographic mass-fragmentographic

method for the assay of PAA in urine. Unfortunately, due to its low concentration in plasma, CSF, and brain tissue the urine method, without major modifications, cannot be applied to these media. To deal with this, Dr. Karoum and associates have employed a highly reproducible method for assaying both free and conjugated PAA in human plasma and monkey CSF.

In humans most PAA is excreted conjugated to glutamine as phenylacetylglutamine (PAG). The percent of total plasma PAA that is conjugated, according to a study of a small group of subjects, is about 20%. We observed, however, a percentage considerably higher than 20%.

Phenylethylamine (PEA)

There are a great number of hypotheses regarding the origin of schizophrenia. Equal or greater in number than the hypotheses of schizophrenia's causes are the findings in schizophrenia that are frequently difficult to reproduce and seemingly unrelated. It occurred to us that two biochemical findings that have now been reproduced in several studies might be related to each other in a cause-and-effect manner. Four groups have found that norepinephrine (NE) concentrations are elevated in the brains, especially the nucleus accumbens, of paranoid schizophrenic patients. While early studies of the endogenous amphetamine-like agent phenylethylamine (PEA) in the urine of schizophrenics produced confusing results, a recent examination of 24-hour urines collected in Washington, D.C. and in Bombay, India indicate that PEA was elevated in paranoid schizophrenic patients compared with non-paranoid patients and normal controls. To test if there could be a linkage between brain NE and elevated PEA concentration, Dr. Karoum and colleagues measured brain catecholamine concentration of rats chronically treated with PEA.

The results of the acute administration of PEA and amphetamine showed, phenylethylamine, in contrast to amphetamine, significantly reduced the norepinephrine concentration in the hypothalamus, but had no effect on hypothalamic dopamine. In the caudate nucleus, both drugs increased norepinephrine and dopamine content. Phenylethylamine in contrast to amphetamine, did not change the concentrations of 3-methoxy-4-hydroxyphenylglycol (MHPG) or 3,4-dihydroxyphenylacetic acid (DOPAC), the major metabolites of norepinephrine and dopamine in the brain. Therefore, although both PEA and amphetamine influence hypothalamic and caudate nucleus catecholamines, the changes produced are not always parallel. Amphetamine is believed to elevate brain catecholamines by stimulating their release as well as inhibiting their re-uptake. Whether or not these same mechanisms are also responsible for the acute effects of PEA on brain catecholamines cannot be discerned from this study. Acute administration of PEA and amphetamine produce similar but not identical behavior in animals.

The most prominent changes of chronic PEA administration was an increase in the norepinephrine concentration of the hypothalamus and nucleus accumbens. There was a small but insignificant norepinephrine increase in the caudate nucleus, but none in the other brain areas. The increase in hypothalamic norepinephrine was accompanied by a similar increase in MHPG and is consistent with the notion of increased norepinephrine turnover in the hypothalamus. The concentration of MHPG in the nucleus accumbens did not change with chronic PEA treatment. Furthermore, the changes in norepinephrine and MHPG concentrations in the hypothalamus and norepinephrine in the nucleus accumbens returned to normal levels within 24 hours after stopping chronic PEA treatment.

The increase in norepinephrine content in the nucleus accumbens is not readily explained by a single phenomenon. On the contrary, it may be induced by a number of effects which either act individually or in concert to culminate in an elevation of norepinephrine content without altering its metabolism. Some of these effects may include increased synthesis and storage of norepinephrine without changing firing by noradrenergic terminals in the nucleus accumbens, increased norepinephrine release, reduced norepinephrine re-uptake, and finally increased norepinephrine turnover with mild inhibition of monoamine oxidase.

Compared with norepinephrine, a relatively small change in dopamine concentration and metabolism was observed after chronic PEA administration. There was a tendency for an increase in dopamine concentration in the hypothalamus, nucleus accumbens and caudate, but these changes failed to reach statistical significance. In all three areas, however, DOPAC was significantly increased. Neither dopamine nor DOPAC was changed in the olfactory tubercle, or the frontal cortex by chronic PEA administration.

In conclusion, we have produced in the rat brain changes in catecholamines, especially norepinephrine, similar to those reported in the brain of chronic paranoid schizophrenics, by subjecting these animals to chronic PEA treatment. Until further direct evidence in paranoid schizophrenics is forthcoming, the possibility of a causative linkage in that disorder must be regarded as tentative. Dr. Zametkin and colleagues examined PEA's relationship to another disorder, attention deficit syndrome.

The clinical efficacy of AMPH and methylphenidate, a cyclized AMPH derivative in the management of Attention Deficit Disorder with Hyperactivity (HAC) has been well established in numerous studies. Also both drugs have been reported to elevate brain concentration of PEA in animals. Because of the parallels in both behavioral and pharmacological effects of the two medications, as well as their structural similarity to PEA, we hypothesized that (a) some alteration in PEA metabolism is present in HAC and/or that (b) the effect of stimulants on PEA may mediate or contribute to the observed therapeutic effect. In that the animal models demonstrate that AMPH elevates brain concentrations of PEA and its urinary excretion, the possibility exists that PEA may mediate the central response to AMPH. Dr. Zametkin and colleagues therefore designed a study to quantify the effects of AMPH on PEA excretion in HAC and to relate any such changes to behavioral response.

The study involved 12 male children, age 6-12 years who were evaluated at the National Institute of Mental Health for impulsive and maladaptive social behavior, hyperactivity, and learning disability. All children met the DSM-III criteria for attention deficit disorder with hyperactivity. In addition, two children met diagnostic criteria for Conduct Disorder and three had developmental disabilities (reading).

All hyperactive children demonstrated a robust increase in excretion of phenylethylamine (PEA) after administration of d-amphetamine (d-AMPH) as well as a smaller, but consistent, increase in phenylacetic acid (PAA). This is the first report of increased urinary PEA and PAA excretion following d-AMPH ingestion in humans. The present findings, however, are consistent with the research reported in rabbits and in monkeys.

Since PEA rapidly and efficiently crosses the blood brain barrier, it may not greatly matter when discussing the significance of its overproduction, whether or not it is peripherally or centrally derived. A more important point is to assess the extent to which such an overproduction may influence the behavioral and therapeutic effects of d-AMPH. In

dealing with this issue as it applies to this study, it should be remembered that PEA and AMPH share many behavioral responses, pharmacological and electrophysiological properties. The increased excretion of PEA produced by d-AMPH in the HAC may complement or perhaps replace some of the responses initially triggered by AMPH. This latter possibility is suggested from the commonly observed absence of behavioral tolerance in HAC to the therapeutic benefits of AMPH. In fact, in most children, the same dose of d-AMPH may be maintained for periods much longer than two weeks without producing significant decreases in its behavioral effect according to clinical impressions. In direct contrast, animal studies show profound and rapid behavioral tolerance to d-AMPH, but generally not to PEA. In this context, it should be mentioned that the behavioral responses to AMPH in the experimental animal cannot always be extrapolated to humans nor can the mechanism involved be the same; for example, the metabolism of d-AMPH itself is quite species specific. Indeed, as demonstrated here as well as being documented in the literature, d-AMPH produced a calming effect on hyperactive children while in the experimental animal it increases motility.

Because of d-AMPH's ability to release brain catecholamines as well as block their neuronal uptake, AMPH behavioral effects are generally believed to be associated with its influence on central catecholamines. D-AMPH has been shown to lead to a time-dependent decrease in the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) excretion without having any significant simultaneous effect on the dopamine metabolite homovanillic acid (HVA) in HAC. The ability of AMPH, however, to also alter other brain substances such as serotonin, certain polypeptides and as shown here, urinary PEA, indicates there may be a more complex mechanism than previously thought.

In summary a large significant increase in urinary PEA was demonstrated in all children treated with AMPH. From this small group there appears to be no direct relationship between clinical therapeutic response to AMPH and PEA excretion, although the capacity to respond may depend on adequately depressed basal levels of PEA. Whether or not PEA plays a role in the mediation of the therapeutic effect of AMPH remains to be elucidated.

Water Regulation

Disturbances in water regulation, first reported in 1933 are common in psychiatric patients. Usually this involves polydipsia, and the secondary consequences of polyuria, hypertension, hyponatremia and even seizures and death. To assess water balance in schizophrenia we measured urine output in patients admitted to our research wards over the past four years.

Urine excretion was measured over two to three consecutive twenty-four hour periods of 65 schizophrenic patients medication-free or on haloperidol .4 mg/kg for at least two weeks. A number of biological parameters were examined including CT scans, blood pressure, serum electrolytes, and presence or absence of tardive dyskinesia.

The mean urine volume of the medication-free schizophrenics was significantly higher than 31 normal controls. No patients off medication met the criteria for The Syndrome of Inappropriate Antidiuretic Hormone, but three patients were hyponatremic. These patients were all hypertensive and had tardive dyskinesia. When on neuroleptic drugs schizophrenic patients excreted even higher volumes. Neither chronicity, cerebral ventricular size, tardive dyskinesia, nor paranoid subtype appear to influence the urine volume.

We concluded from these findings that schizophrenics frequently have increased urine volumes that appear to be increased further by neuroleptic medication. Occasional patients show evidence of water intoxication. Various biological, and diagnostic parameters do not clarify the etiology of this phenomenon.

Zinc

Although zinc is a trace metal comprising about 2.5 g of total body mass, it is the fourth most prevalent cation (a positively charged atom or group of atoms) in the brain (following sodium, potassium and magnesium). Zinc plays an essential role in several enzyme systems concerned with protein synthesis, DNA replication and repair, and the stabilization of biological membranes. It directly affects synaptic transmission and inhibits the uptake of norepinephrine and choline into brain synaptosomes.

The reason Dr. Potkin and colleagues decided to study this mineral is that zinc has been implicated in several aspects of brain metabolism. Prenatal zinc deficiency in experimental animals reportedly induced aggressiveness and persistent learning disability. A role was proposed for zinc in the development of psychiatric symptoms when high urinary zinc was found in a patient with porphyria. It was hypothesized that increased urinary zinc excretion resulted in zinc deficiency which produced psychiatric symptoms typical of the disease. Subsequently, it was demonstrated that the increased urinary excretion of zinc in patients with porphyria is associated with uroporphyrin binding of zinc. Acute zinc depletion in humans has also been produced by oral administration of the amino acid L-histidine; the emotional lability, ideas of reference, acute depression, and cerebellar dysfunction that accompany the zinc depletion are dramatically reversed within 24 hr of zinc replacement therapy, despite continued administration of L-histidine. Zinc deficiency associated with parenteral hyperalimentation can result in paranoia and confusion, which is also rapidly reversed by zinc replacement therapy. In premature infants, zinc deficiency may also cause irritability and inappropriate crying that the mother cannot stop by means of consoling; oral zinc reverses this irritability and crying, frequently within a few hours of administration.

Zinc deficiency has been hypothesized to be an important factor in the pathogenesis of schizophrenia. A 50% decrease was found in zinc content of the autopsied brains of ten schizophrenic patients when compared with ten non-schizophrenic patients' brains; however, researchers were unable to confirm these findings in autopsied brains from four schizophrenic patients compared with other patients and controls.

Other researchers identified a subgroup of schizophrenic patients (10 to 20%) with low urinary zinc and claimed marked improvement in their psychiatric symptoms with zinc supplements. This clinical benefit attributed to zinc has been difficult to evaluate critically because, among other reasons, oral zinc was only one of the many substances in a complex prescription of vitamins, nutrients, and neuroleptic drugs given to these patients.

We have previously found no significant differences in zinc concentrations in blood, urine, hair, and gastric aspirate in unmedicated schizophrenic patients and our own published controls. Zinc concentrations in hair, blood and urine, however, may not reflect brain or total body zinc status. In an attempt to obtain another index of body zinc more closely related to cerebral zinc metabolism, we measured cerebrospinal fluid (CSF) zinc concentrations in ex-heroin addicts, schizophrenic patients, and normal subjects.

While the ex-heroin addicts studied had significantly lower CSF zinc concentrations than medicated schizophrenic patients or normal controls, we note that none of these patients' CSF zinc concentrations were below the range of the normal controls. Also, only one of the 28 drug-free schizophrenics had a CSF zinc concentration below the control subjects' range.

Because of the marked concentration of white subjects in the drug-free schizophrenic group, we were unable to determine whether the low CSF zinc concentrations in these patients were a function of race, diagnosis, or medication status. The lack of a significant difference of CSF zinc concentration between the black drug-free schizophrenic patients and normal controls argues against a difference based on diagnosis. Further studies to determine the relation of CSF zinc to race (white vs black), diagnosis (schizophrenic vs nonschizophrenic), and medication status (neuroleptic drugs vs no drugs), are required and are important to carry out.

Monoamine Oxidase

The discovery of the monoamines and the development of the hypotheses that they may be involved in a number of neuropsychiatric conditions (including schizophrenia, affective disorders and depression) has led to our three decades of research. Prominent in the investigations of the role of monoamines in neuropsychiatric conditions have been studies of platelet monoamine oxidase (MAO). By 1972, when MAO was first reported to be decreased in platelets of chronic schizophrenic patients, MAO had been widely accepted as an enzyme of major importance to neurotransmitter metabolism.

In the intervening years, patients in our wards have been tested for MAO platelet levels at entry and on follow-up protocols. These long standing protocols are now coming to a close, data collection is being slowed and final analyses are to begin shortly. It is expected that concluding manuscripts will be published by next year's Annual Report.

Alpha-Adrenergic Receptor Function

An alteration in adrenergic neurotransmission has been implicated in the pathophysiology of schizophrenia. One mechanism by which adrenergic activity might be changed is through an alteration in alpha-adrenergic receptor number. As the human platelet has alpha-receptors, it is possible to measure not only the number of alpha-receptors in the platelets of schizophrenic patients, but also to quantify a biological response to alpha-receptor occupancy, the norepinephrine (NE) inhibition of cyclic AMP (cAMP) production. We have also examined alpha-adrenergic function in the platelets of schizophrenic and schizoaffective patients by measuring alpha-receptors and cAMP production.

Results of this study, for the first time, demonstrate an increase in alpha-receptor binding in the platelets of a group of chronic schizophrenic patients. As the affinity of the platelet alpha-receptor is similar in controls and schizophrenic patients, the increase in specific binding of [^3H]DHE to platelet membranes measures an increase in the number of alpha-receptors in these platelets.

It is tempting to speculate about the relevance of these findings to the schizophrenic syndrome. Alpha $_2$ -receptors exert inhibitory control over several functions in the rat brain. If neurons, like platelets, have more alpha-receptors in schizophrenic patients, and if the neurons inhibit inhibitory brain centers, perhaps an increase in the number of central alpha $_2$ -receptors contribute to some of the symptoms that characterize many schizophrenic

patients. No correlation, however, was found between platelet alpha-receptor number and items or symptoms measured on the Brief Psychiatric Rating Scale (BPRS). Also, the previous report of an increased number of alpha-receptors in paranoid patients was not confirmed.

This study does, however, replicate the previous finding of decreased PGE₁-stimulated cAMP production in platelets from male schizophrenic patients and extends the findings to include platelets from female schizophrenic patients as well. Decreased cAMP production in platelets from schizophrenic patients of both sexes has been previously reported. Diminished adenylate cyclase activity in platelet lysates from schizophrenic patients probably plays a major role in the decreased production of cAMP. As the control of cAMP production in the neuroblastoma-glioma cell resembles that in the platelets, neurons as well as platelets in schizophrenic patients might produce less cAMP. As decreased cAMP production leads to diminished protein phosphorylation and an accompanying loss of cellular protein activation, decreased cAMP production may alter brain neurotransmission. Interestingly, the cAMP concentration in the CSF of some schizophrenic patients was shown to be decreased.

In conclusion, this study finds two abnormalities in the platelets of chronic schizophrenic patients. It remains for future research to determine how a finding of an increase in the number of alpha-adrenergic receptors and a decrease in the PGE₁-stimulated cAMP production will contribute to a better understanding of the phenomenology of schizophrenia.

Calcitonin

The final piece of work in the psychopharmacology section concerns calcitonin. Calcitonin is a peptide hormone secreted by the C-cells of the thyroid gland. Its primary physiological effect is to decrease plasma calcium and phosphorus concentrations. This action of calcitonin is particularly pronounced whenever plasma calcium becomes elevated. Calcitonin was discovered in 1962, and it was found that perfusion of the thyroid-parathyroid complex with hypercalcemic solutions caused a lowering of plasma calcium concentrations. These results could not be explained in terms of decreased secretion of parathyroid hormone, which increases plasma calcium. Thus, it eventually became established that the thyroid secretes calcitonin, a hormone that decreases plasma calcium.

The two primary sites of action of calcitonin are bone and kidney. In bone, calcitonin inhibits bone resorption and calcium release. In kidney, calcitonin decreases tubular reabsorption of calcium, phosphorus, and other electrolytes. Calcitonin, however, may have additional sites of action: Calcitonin increases protein synthesis in the intestine, and decreases protein synthesis in the pancreas. Systemically-administered calcitonin accumulates in muscle and blood vessel walls, as well as in kidney and bone. Recently, a substantial literature has begun to accumulate suggesting that the brain is another major site of action of calcitonin.

Is The Brain A Target Organ For Calcitonin?

There have been reports that calcitonin is present in human cerebrospinal fluid as well as in the brain parenchyma. It has been established that calcitonin is present in the pituitary gland. Whether brain and cerebrospinal fluid calcitonin is thyroid-derived is unknown; however, pituitary calcitonin is not decreased by thyroidectomy, and is therefore thought to be produced by the pituitary. Secretion of pituitary calcitonin has not been demonstrated.

One action of calcitonin that may be mediated by the brain is inhibition of gastric acid secretion. A number of studies have shown that calcitonin inhibits secretion of gastric acid when administered peripherally. Recently, it has been reported that intracerebral administration of salmon calcitonin in dosages as small as .0002 units decreased gastric acid secretion. In contrast, approximately 1000-fold larger dosages were required to inhibit gastric secretion by subcutaneous administration. Therefore, inhibition of gastric acid secretion may be another effect of calcitonin that is mediated by the central nervous system.

Researchers have recently demonstrated that gastric acid secretion can be stimulated by intrahypothalamic injections of small amounts of gastrin. This finding suggests that the central nervous system control of gastric acid secretion is localized in the hypothalamus. If so, the hypothalamus may also be the site at which calcitonin acts to inhibit gastric acid secretion.

To test the possibility that calcitonin is involved in the regulation of feeding behavior, we investigated the effects of calcitonin injections on eating in rats. Initially, we studied the effects of subcutaneous salmon calcitonin on 24 hour food and water intake. Rats were given single subcutaneous injections of synthetic salmon calcitonin, and their eating, drinking, urine and fecal excretion, and weights were measured for the following 24 hours. Calcitonin produced a dose-dependent inhibition of eating, resulting in a 40% decrease in food intake with a dose of 50 medical research council units (units or U) per kg. There was a concomitant decrease in fecal excretion and body weight, all of these effects recovering over the succeeding 24 hours.

Calcitonin did not significantly influence drinking, but did increase urine output. This increased urine output was followed, from 24-48 hours after calcitonin injections, by an increase in drinking which was apparently compensatory to the increased urine excretion. It has previously been reported that calcitonin increases urine volume and excretion of sodium and other electrolytes. Calcitonin did not produce diuresis in a study of marsh mice but did decrease food intake in these animals.

The fact that calcitonin was effective over an entire 24 hour period suggested that calcitonin is capable of inhibiting eating for at least several hours after injection. We found, also, that calcitonin decreases drinking under conditions of food deprivation and appears to have a direct inhibitory effect on drinking in rats.

These results suggest that calcitonin can act directly on the central nervous system to inhibit eating. A substantial decrease in food intake is produced by small dosages of calcitonin and this effect depends upon the structural integrity of the calcitonin molecule. Stress-induced eating, as well as spontaneous eating, is inhibited by calcitonin. Human calcitonin also inhibits eating, in a dosage range that is consistent with its potency, when appropriate testing conditions are employed. Therefore, calcitonin has a general inhibitory effect on eating through a direct action on the central nervous system.

Does Calcitonin Produce Illness?

The question arises as to whether the inhibition of eating by calcitonin is a specific, direct effect on eating, or is secondary to some other process such as sedation or production of illness or general malaise.

Calcitonin has been reported to cause a decrease in the activity of rats. This is probably not an explanation for the anorectic effects of calcitonin, because sedative drugs generally stimulate eating, while eating is inhibited by stimulants such as amphetamine.

A question that is more difficult to answer is whether calcitonin produces nausea or illness which, in turn, inhibits eating. We are convinced that this is not the case because: (i) Food-deprived rats that had received calcitonin injections which inhibits eating invariably began to eat rapidly and avidly, and did not exhibit any grossly-observable signs of illness and nausea while eating. (ii) Psychiatric patients receiving calcitonin seldom reported nausea, and the literature on administration of calcitonin to humans does not reveal a significant incidence of nausea and illness. (iii) Calcitonin did not act as an aversive stimulus in rats, in terms of producing a conditioned taste aversion.

The primary function of calcitonin is currently thought to be in the regulation of plasma calcium. Increases in plasma calcium result in large increases in calcitonin secretion. However, a variety of foodstuffs, such as triglycerides, also increase calcitonin secretion. In rats, triglycerides actually stimulate calcitonin secretion to a greater degree than does an increase in plasma calcium. In addition, several hormones (glucagon, cholecystokinin, and gastrin) known to be released in response to food intake also stimulate the release of calcitonin. Recent evidence suggests that some of these hormones may exert an anorectic effect in addition to their well-established roles in metabolic processes. On the basis of these observations, it was hypothesized that the anorectic effects of these hormones and of certain foods is mediated by calcitonin. In other words, increased plasma calcitonin concentrations resulting from food intake and subsequent increases in hormone secretion might exert a negative feedback effect on subsequent food intake.

Experiments performed to test this hypothesis have indeed shown that calcitonin has anorectic properties. Salmon calcitonin is a potent and long-lasting anorectic agent in rats and in monkeys when injected subcutaneously. When injected intracerebrally calcitonin decreases spontaneous eating in rats in dosages as small as 0.2 units. Others have reported that tail-pinch induced eating in rats can be decreased by calcitonin in dosages as small as 0.002 units. Subcutaneous injections of calcitonin also caused a transient loss of body weight and a pronounced diuresis. Intracerebral injections of calcitonin caused a similar loss of body weight but no diuresis. This suggests that the body weight loss that is produced by subcutaneous calcitonin is caused by decreased food intake rather than uncompensated diuresis.

Human calcitonin, the least-potent form of calcitonin known, was found to be capable of inhibiting eating in rats on a short-term basis. Patients who had received synthetic salmon calcitonin in the course of other experiments had a slight decrease in body weight the day after calcitonin injections. But effects of calcitonin on eating behavior have not been directly studied in man.

One study has raised the interesting possibility that altered calcitonin production or secretion may be associated with some disorder of eating behavior or appetite. It was found that obese rats had increased concentrations of calcitonin in their thyroid and pituitary glands, but not in serum. Because serum calcitonin tended to be higher, not decreased, in the obese animals, the role of calcitonin in the development of this obesity is unclear. Calcitonin concentrations in serum from anorexia nervosa patients were measured, but were found to be normal. The possibility that calcitonin is associated with these and other disorders of eating or appetite, such as cancer cachexia, still exists and may warrant further investigation.

At the present time, the mode of action of calcitonin on the central nervous system is unknown. On the basis of studies in mouse Ehrlich ascites tumor cells, it has been suggested that the primary effect of calcitonin is to promote redistribution of calcium ions between cellular and extracellular compartments. It has recently been reported that calcitonin reduces *in vitro* uptake of calcium into hypothalamic explants. Thus, calcitonin may also alter cellular distributions of calcium in the brain.

Therefore, calcitonin is a potent inhibitor of eating behavior and appears to produce this effect through an action on the central nervous system. A number of other recently-discovered effects of calcitonin, including suppression of prolactin secretion, inhibition of gastric acid secretion, and possibly the induction of analgesia, also seem to be mediated by the central nervous system. Undoubtedly, calcitonin has other as yet undiscovered effects on the central nervous system as well. The parameters of the effects of calcitonin on eating, prolactin secretion, and other functions suggest that calcitonin will prove to be an interesting and useful psychopharmacological agent.

The Viral Hypothesis Of Schizophrenia

History is replete with examples suggestive of associations between outbreaks of infectious disorders and psychoses. Reports from ancient and prerennaissance times indicate coincidence of epidemic illnesses (e.g. plague) with dementia-like disorders. Others such as Benjamin Rush and Karl A. Menninger, also, connected psychosis with influenza outbreaks.

To complicate matters further, there are several examples of known viral diseases that are, at least initially, diagnostically similar to schizophrenia. The rare Russian tick-born encephalitis, endemic to the Yakut Republic of the USSR, is said to be, in its chronic form, indistinguishable from classical schizophrenia. Other, more common viral encephalitides are also either initially confused with schizophrenia or develop post-encephalitic dementia-like sequelae. It was a Russian investigator, who first proposed that a virus, present in the body for a long time in a latent state, could produce symptoms of schizophrenia. Subsequent Russian literature, particularly in the late 1950's to 1960's, focused heavily on a viral etiology of schizophrenia.

Recent establishment of the existence of "slow viral infections" (viruses having an incubation period of years to decades prior to producing symptoms) have led a few Western researchers to propose an etiologic similarity between neurodegenerative disorders and schizophrenia. The first human slow virus disease discovered was Kuru, a neurological disorder endemic to New Guinea and transmitted by cannibalism. Kuru, however, is unlike schizophrenia because it results in massive brain degeneration and ultimately death. Other diseases, also thought to be of slow-virus origin, such as Creutzfeld-Jacob disease and perhaps even multiple sclerosis, have clearly associated neurodegenerative changes. In addition, some conventional viruses, such as measles, can also produce neurodegenerative disorders, such as subacute sclerosing panencephalitis, many years after the initial contact.

Indirect support for the viral hypothesis has come from epidemiological studies of schizophrenia. The seasonality of schizophrenic births, a peak in late winter and early spring that coincides with the peak incidence of some viral infections, such as measles and rubella, has been described. The uneven prevalence of schizophrenia throughout the world, also, is a pattern similar to the occurrence of some known viral diseases.

Further support for this hypothesis has grown out of the application of specific immunological techniques. Serum and CSF immunoglobulins, often elevated in viral

diseases, have been found in some studies to be increased in schizophrenic patients. This finding is not, however, consistent and the type of immunoglobulin elevated varies. Further studies of viral specific antibodies support the possibility that these elevations are a consequence of viral infection. Increased serum antibody titers to herpes simplex type 1 virus were reported in one study, although not subsequently confirmed. In addition, an elevation of the ratio of CSF to serum IgG antibody titers has been reported for both measles and cytomegalic (CMV) virus.

In recent work in our laboratory, Dr. Janice Stevens examined histologic sections from a number of brain regions from 38 schizophrenic and 28 non-schizophrenic patients, all between ages 21 and 54. Three quarters of the brains from the schizophrenic patients had patchy fibrillary gliosis most marked in the periventricular, and periaqueductal structures, as well as basal forebrain. Gliosis and other microscopic changes were equally evident in the control brains, although occurring in different regions. The finding of gliosis in limbic related structures in the schizophrenic patients is consistent with earlier neuropathological work as well as with the enlarged ventricles found in some schizophrenic patients and is further evidence consistent with viral produced changes.

In subsequent preliminary work, Dr. Stevens and colleagues attempted to identify specific viruses likely to be associated with the gliosis. Brains from patients with a chronic schizophrenic illness were immunoreactive to specific CMV antigens, while they were non-reactive to herpes simplex antigens. Although the reaction was also present in three of thirteen controls, in the controls the reaction was shown to be nonspecific, having cross-reactivity with other antigens. While much further work is required, this is an exciting new research direction that will demand increasingly broader attention.

Blinking

Since the cornerstone of neurochemical research in schizophrenia remains the dopamine hypothesis, the lack of an easily quantifiable and readily available clinical measure of central dopaminergic activity particularly hampers neurochemical research in this direction. Investigations of the concentrations of dopamine and its metabolites in the cerebrospinal fluid (CSF), or of hormonal indicators of central dopamine activity, such as prolactin, have failed to confirm that schizophrenic patients have elevated central dopaminergic activity. Even post-mortem studies, which show increased dopamine receptors in schizophrenic brains are equivocal, as this difference may be caused by neuroleptic therapy.

A promising tool in this area appears to be spontaneous eye blink rates. Decreased blinking in Parkinson's disease, a condition associated with decreased central dopaminergic activity, and increased blinking in schizophrenia, suggests that blink rates are positively correlated with central dopaminergic activity. Also, apomorphine, a dopamine agonist, increases blinking in monkeys. Therefore, blinking could be a useful monitor of the functional state of this neurotransmitter. Moreover, blinking is readily quantified and can be observed even in the most uncooperative patients. To test a series of hypotheses concerning the behavior of spontaneous eye blinks in schizophrenia, based primarily on the dopamine hypothesis, Dr. Karson and colleagues studied 34 male and 10 female chronic schizophrenic patients.

Results showed that the mean blink rate for medication free patients significantly exceeded that of normal controls, suggesting that blink rates in schizophrenic patients are not normally distributed, and that neuroleptic treatment reduced mean blink rates. In another study with 24 male and seven female chronic schizophrenic patients, a significant

correlation was demonstrated between blink rates and monoamine oxidase activity in drug-free patients.

Looking at blink rates and ventricular size, if blink rates are a dopamine mediated parameter and dopaminergic activity is increased in normal ventricle schizophrenic patients, then blinking may be increased in this group of patients in contrast to large ventricle schizophrenic patients. Moreover, blink rates of normal ventricle patients should be decreased by neuroleptics because of their dopamine blocking properties. A neuroleptic effect on blinking may not occur in large ventricle patients as has been the case with clinical responsiveness.

In examining 34 chronic schizophrenic patients, Dr. Karson and colleagues found that schizophrenic patients with normal ventricles had increased blink rates that were reduced by neuroleptics. This does not appear to be the case in schizophrenic subjects with large ventricles. The question arises as to what is the normal response of blink rates to neuroleptics. Although the effect of neuroleptics on blink rates in normals has not been studied, blink rates in rhesus monkeys are reduced by haloperidol. The blink rate response to neuroleptics of large ventricle schizophrenic patients appears to be different, a finding which is consistent with their poor clinical response to neuroleptics.

Thus, clinical response to neuroleptics, the relationship between psychotic symptoms and plasma prolactin concentrations, and blink rates on and off neuroleptics support the hypothesis that ventricular size is a meaningful factor in subtyping the schizophrenic syndrome. In so far as blinking and these other relationships are dopamine mediated it appears that the dopamine hypothesis of schizophrenia is most relevant in those patients with normal ventricles and high blink rates.

To summarize these and our other findings on blinking in schizophrenia:

- (1) Schizophrenic patients have elevated blink rates which are reduced by neuroleptics.
- (2) Haloperidol-induced reductions in blink rates correlate with reductions in florid psychotic symptoms, particularly in patients with normal VBR.
- (3) Platelet MAO correlates inversely with blink rates, but not in patients with TD.
- (4) Two groups of patients who may have altered responses to neuroleptics, namely patients with large VBR, and patients with tardive dyskinesia, have blink rates comparable to other schizophrenic patients. Neuroleptics, however, do not decrease the blink rates of either group.
- (5) Patients with impaired cognitive function, as measured by the Halstead-Reitan battery, have decreased blinking as compared to unimpaired patients.
- (6) There is no evidence that serotonin or noradrenergic influence blink rates.

Finally, spontaneous eye-blink rates are readily observed and easily quantified. They are linked to the activity of the brain's dopaminergic systems, as well as to ongoing mental activity. In two diseases that are postulated to involve abnormal dopaminergic activity, Parkinson's disease and schizophrenia, blink rates are not only altered, but are related to subtle variations in the status of the illness. The severity of impaired movement, TD, impaired cognitive function, and abnormal CT scan findings, are associated with altered properties of spontaneous blinking even within their respective disease. Perhaps in the future, spontaneous eye-blink rates will serve as the "pulse" of the brain, through which the status of thought processes or neurochemical activity will become accessible to the outside observer.

Multiple Personality Disorder

The concluding entry into this section of the Annual Report pertains not to schizophrenia but to another disorder: multiple personality disorder syndrome. To investigate and rigorously document the clinical and physiological phenomena, Dr. Frank Putnam and colleagues are pursuing the disorder from three avenues.

- (1) The first approach utilizes repeated neurophysiological measures across alternate personalities which are compared to control subjects simulating this condition.
- (2) The second line of inquiry is the establishment of a large scale data base of cases collected in a standardized manner. A 24 page questionnaire is used to collect data on: presenting symptoms, past psychiatric, family, social, childhood and educational history; method of diagnosis, phenomenology of the alternate personalities, treatment and outcome.
- (3) The third avenue of investigation is prospective studies on a cohort of patients undergoing treatment around the United States. Several cases of multiple personality disorder in children and adolescents are included in this cohort of patients.

Subjects are patients who meet DSM III criteria for multiple personality disorder and are admitted as outpatients to the NIH clinical center. These patients serve as subjects in the physiological and psychological investigations. Clinical center normal volunteers and professional actors from the Psychodrama Institute located at Saint Elizabeths Hospital in Washington, D.C. serve as control subjects for the physiological and psychological studies.

Drug-free subjects undergo a repeated series of EEG and visual evoked potential studies, galvanic skin response (GSR) and other autonomic measures are being studied across alternate personality states and simulating controls. Cerebral blood flow using the xenon inhalation technique is being studied across alternate personality states tested under two conditions: a resting study and an activated state using an automated version of the Wisconsin Card Sort. Professional actors simulating multiple personality patients are serving as controls.

A 24 page (186 item) questionnaire developed and piloted during 1981-1982 has been distributed to clinicians around the United States who are engaged in treating patients with multiple personality disorder. Presently, over 150 cases have been collected and analyzed with this form. A very large scale sampling questionnaire is currently under development which will be used to determine the incidence and prevalence of multiple personality disorder in the United States.

The first prospective study involves the follow-up of a cohort of adult multiple personality patients undergoing a variety of treatment modalities. These patients were all screened with a standardized, videotaped interview and are being followed at one year intervals.

The second group of patients involved in prospective studies are children identified by two local sexual abuse agencies -- The Prince Georges County Sexual Assault Center and the Montgomery County Protective Services. These children fit a profile developed through the questionnaire study and a literature review and are followed by social workers in the respective agencies. The focus on sexual abuse derives from the questionnaire study data showing that 83% of adult multiple personality patients suffered sexual abuse as children.

To date, findings show that visual evoked potentials demonstrate personality specific changes which are not matched by simulating control subjects. Power spectral analysis of the EEG data show statistically significant differences in the high frequency Beta waves across alternate personalities that are not produced by simulating control subjects. Proactive inhibition memory testing reveals a statistically significant higher discrimination of set in multiple compared to simulated controls.

An analysis of 100 current cases of multiple personality disorder which were collected independently of each other reveals a high degree of similarity in symptoms and alternate personality structures across patients. The most striking finding is the high degree of reported child abuse suffered by these patients. Outcome data suggests that psychotherapy and hypertherapy are the most effective therapeutic interventions.

No findings are currently available at this time on the prospective studies. Approximately 30 patients and therapists are participating in the adult study and eight children and adolescents are involved in the child study.

At this point the physiological studies confirm the anecdotal reports of clinicians over the last 150 years who noted physiological differences across alternate personality states. These replicable physiological changes may provide clues as to the interaction of personality and physiology which will be useful in understanding psychosomatic processes.

The questionnaire data is providing the first large scale data on the nature of this disorder and demonstrates that there is a high degree of similarity across cases for most phenomena of this disorder. The linkage to child abuse has often been suspected, but the questionnaire data confirms that multiple personality disorder is highly correlated with child abuse.

Methods

Appropriate methods are employed and where possible, studies are performed in a double-blind design. Also whenever possible, sample sizes are large enough to allow for general conclusions.

Significance to Biomedical Research and the Program of the Institute

Schizophrenia affects approximately one percent of the population, or about 2.2 million persons in this country. The 1976 figures show a direct or indirect cost to the nation of about 20 billion dollars. This is about the same cost for cancer, according to figures released by the National Cancer Institute. Given the number of individuals afflicted with this condition and the high cost to the nation, our research into potential etiologies, prevention and treatment of this disorder are highly significant.

Our research into the schizophrenias spans and connects a wide range of psychiatric subspecialties. Our biochemical studies are focused, primarily, on elucidating biological markers for the disease. To this end, for example, we have been finding elevated phenylethylamine (PEA) concentrations in a naturally occurring amphetamine-like substance in several national, as well as cross-culturally studied, populations.

Our neuropsychiatry unit has produced important neuroanatomical insights into the disorder, as well. Using computerized axial tomography (CT) scans, and building on our laboratory's previous finding of enlarged cerebral ventricles in the brains of some schizo-

phrenic patients, we are now correlating ventricular size with a wide array of other measures. These include poor premorbid history, antipsychotic drug response, spontaneous eye blink rates, age, duration of illness and at autopsy, evidence of catecholamine production in the brain. Our CT scan work has been repeatedly cited in the literature and multiple laboratories are beginning to use ventricular size as a measure for subgrouping schizophrenic patients.

Our research into possible etiological factors has also produced innovative histological findings. It appears, in preliminary work, that we have found evidence of cytomegalovirus in several limbic region sections of the brains of some schizophrenic patients. These findings provide tentative support for the viral hypothesis of schizophrenia, which has been only indirectly supported, up until now, by epidemiological studies.

And finally, we are continually reassessing all of our schizophrenia research with an eye to developing a new method of subtyping schizophrenic patients. To date, patients have been divided according to the overt behavioral manifestations of their individual psychoses. We are beginning to move towards a new typology, one, perhaps, better organized along physiological, neuroanatomical and pharmacologically responsive dimensions. By developing a new classification system, it is hoped that new perspectives on research into etiologies, prevention and treatments will result.

Proposed Course

We plan to continue examining the schizophrenia syndrome from a multi-disciplinary perspective. To this end, we will continue to search biochemically for markers through the elucidation of abnormalities in the production and function of catecholamines, enzymes and hormones. We plan to continue refining our ability to assess neuroanatomical and metabolic findings derived from such technological innovations as computerized axial tomography (CT) scans, cerebral blood flow and positron emission tomography scans. We plan to continue our etiological investigations into a possible viral component of the disorder. And in a manner that coherently connects this diverse body of research, we will continue to work towards a more productive typology of the schizophrenia syndrome.

Publications

Karoum, F., Chuang, L-W., Eisler, T., Calne, D.B., Liebowitz, M., Quitkin, F.M., Klein, D.F. and Wyatt, R.J.: Metabolism of (-)deprenyl to amphetamine and methamphetamine may be responsible for deprenyl's therapeutic benefit: A biochemical assessment. Neurology 32: 503-509, 1982.

Weinberger, D.R., DeLisi, L.E., Perman, G.P., Targum, S. and Wyatt, R.J.: Computed tomography in schizophreniform disorder and other acute psychiatric disorder. Archives of General Psychiatry 39: 778-783, 1982.

Jeste, D.V., Linnoila, M., Wagner, R.L. and Wyatt, R.J.: Serum neuroleptic levels and tardive dyskinesia. Psychopharmacology 76: 377-380, 1982.

Jeste, D.V. and Wyatt, R.J.: Therapeutic strategies against tardive dyskinesia--Two decades of experience. Archives of General Psychiatry 39: 803-816, 1982.

Karson, C.N., Bigelow, L.B., Kleinman, J.E., Weinberger, D.R. and Wyatt, R.J.: Haloperidol induced changes in blink rates correlate with changes in BPRS score. British Journal of Psychiatry 140: 503-507, 1982.

Luchins, D.J., Weinberger, D.R. and Wyatt, R.J.: Schizophrenia and reversed cerebral asymmetry in schizophrenia. American Journal of Psychiatry 139: 753-757, 1982.

Luchins, D.J., Weinberger, D.R., Torrey, E.F. and Wyatt, R.J.: HLA and reversed cerebral asymmetries: Possible markers for a subgroup of schizophrenics. In: Usdin, E and Hanin, I. (eds.). Biological Markers in Psychiatry and Neurology, New York, Pergamon Press, 1982, pp. 485-493.

Karson, C., Freed, W.J., Bigelow, L.B., Weinberger, D.R. and Wyatt, R.J.: Blink rates in schizophrenia. In: Usdin, E. and Hanin, I. (eds.). Biological Markers in Psychiatry and Neurology, New York, Pergamon Press, 1982, pp. 339-345.

Potkin, S.G., Jeste, D.V., Karoum, F., Doongaji, D.R., Apte, J.S., Sheth, A.S., Chuang, L-W. and Wyatt, R.J.: A cross-cultural design to test a biological hypothesis of schizophrenia. In: Usdin, E. and Hanin, I. (eds.). Biological Markers in Psychiatry and Neurology, New York, Pergamon Press, 1982, pp. 49-59.

Kleinman, J.E., Weinberger, D.R., Rogol, A., Shilling, D.J., Mendelson, W.B., Davis, W.E., Bunney, W.E. Jr. and Wyatt, R.J.: Naloxone in chronic schizophrenics patients: Neuroendocrine and behavioral effects. Psychiatry Research 7: 1-7, 1982.

DeLisi, L.E., Goodman, S., Neckers, L.M. and Wyatt, R.J.: An analysis of lymphocyte subpopulation in schizophrenic patients. Biological Psychiatry 17: 1003-1009, 1982.

Karson, C.N., Bridge, T.P., Phelps, B.H., Wise, C.D., Potkin, S.G., Apostoles, P.S. and Wyatt, R.J.: The effect of oral glucose on platelet monoamine oxidase. Biological Psychiatry 17: 1011-1015, 1982.

Weinberger, D.R., Rogol, A.D., Bigelow, L.B., Klein, S.T., Gillin, J.C. and Wyatt, R.J.: Plasma prolactin concentrations and psychopathology in chronic schizophrenia. Archives of General Psychiatry 39: 655-657, 1982.

Cannon-Spoor, H.E., Potkin, S.G. and Wyatt, R.J.: Measurement of premorbid adjustment in chronic schizophrenia. Schizophrenia Bulletin 8: 470-484, 1982.

Jeste, D.V. and Wyatt, R.J.: Guest Editorial: Neuroleptics and tardive dyskinesia: Quo vadis? Journal of Clinical Psychopharmacology 2: 303-304, 1982.

Wagner, R.L., Jeste, D.V., Phelps, B.H. and Wyatt, R.J.: Enzyme studies in tardive dyskinesia. I. One-year biochemical follow-up. Journal of Clinical Psychopharmacology 2: 312-314, 1982.

DeLisi, L.E., Jeste, D.V., Phelps, B.H. and Wyatt, R.J.: Enzyme studies in tardive dyskinesia. II. Familial aspects. Journal of Clinical Psychopharmacology 2: 315-317, 1982.

Jeste, D.V., Linnoila, M., Fordis, C.M., Phelps, B.H., Wagner, R.L. and Wyatt, R.J.: Enzyme studies in tardive dyskinesia. III. Noradrenergic hyperactivity in a subgroup of tardive dyskinesia patients. Journal of Clinical Psychopharmacology 2: 318-320, 1982.

Potkin, S.G., Shore, D., Torrey, E.F., Weinberger, D.R., Gillin, J.C., Henkin, R.I., Agarwal, R.R. and Wyatt, R.J.: Cerebrospinal fluid concentrations in ex-heroin addicts and patients with schizophrenia: Some preliminary observations. Biological Psychiatry 17: 1315-1323, 1982.

Gillin, J.C., Carpenter, W.T., Hambridge, K.M., Wyatt, R.J. and Henkin, R.I.: Zinc and copper in patients with schizophrenia. L'Encephale VIII: 435-444, 1982.

Weinberger, D.R. and Wyatt, R.J.: Brain morphology in schizophrenia: In vivo studies. In: Henn, F.A. and Nasrallah, H.A. (eds.). Schizophrenia as a Brain Disease, New York, Oxford University Press, 1982, pp. 148-175.

Kleinman, J.E., Karoum, F., Rosenblatt, J.E., Gillin, J.C., Hong, J., Bridge, T.P., Zalcman, S., Storch, F., del Carman, R. and Wyatt, R.J.: Postmortem neurochemical studies in chronic schizophrenia. In: Usdin, E. and Hannin, I. (eds.). Biological Markers in Psychiatry and Neurology, New York, Pergamon Press, 1982, pp. 67-76.

Karson, C.N., Weinberger, D.R., Bigelow, L.B. and Wyatt, R.J.: Clonazepam treatment of chronic schizophrenia: Negative results in a double-blind placebo-controlled trial. American Journal of Psychiatry 139: 1627-1628, 1982.

Freed, W.J. and Zec, R.F.: Criteria for ruling out sedation as an interpretation of neuroleptic effects. Behavioral and Brain Sciences 5: 57-59, 1982.

Stevens, J.R.: Editorial: Neuropathology of schizophrenia. Psychological Medicine 12: 695-700, 1982.

Stevens, J.R.: Neuropathology of schizophrenia. Archives of General Psychiatry, 39: 1131-1139, 1982.

Weinberger, D.R.: Computer tomography (CT) findings in schizophrenia: Speculation on the meaning of it all. Journal of Psychiatric Research (in press).

Wagner, R.L. and Weinberger, D.R.: Recent neuropathological observations in schizophrenia. In: Bunney, W.E., Ingvar, D.H. and Usdin, E. (eds.). Local Cerebral Metabolism Position Emission and X-Ray Tomography in Psychiatry and Neurology, Boxwood Press, Pacific Grove (in press).

Weinberger, D.R.: Implications of cerebral ventricular size for response to neuroleptic therapy in schizophrenia. In: Garfinkel, P.E. (ed.). Guidelines for Use of Psychotropic Drugs, Spectrum (in press).

Weinberger, D.R.: The role of x-ray computed tomography in clinical psychiatry. In: Hall, R.C.W. and Beresford, T.P. (eds.). Handbook of Psychiatric Diagnostic Procedures, Spectrum (in press).

Nasrallah, H.A., Rivera-Calimlim, L., Rogol, A.D., Gillin, J.C. and Wyatt, R.J.: Fluphenazine decanoate: Plasma concentrations and clinical response. Psychopharmacology Bulletin (in press).

Potkin, S.G., Cannon-Spoor, E., DeLisi, L.E., Neckers, L.M. and Wyatt, R.J.: Phenylalanine, tyrosine and tryptophan metabolism in schizophrenia. Archives of General Psychiatry (in press).

Kleinman, J.E., Karson, C.N., Weinberger, D.R., Freed, W.J., Berman, K.F. and Wyatt, R.J.: Eye-blinking in chronic schizophrenic patients grouped by cerebral ventricular size. American Journal of Psychiatry (in press).

DeLisi, L.E. and Wyatt, R.J.: Abnormal immune functioning in schizophrenic patients. Psychopharmacology Bulletin (in press).

Freed, W.J., Bing, L.A., Anderson, A. and Wyatt, R.J.: Calcitonin as an anorectic agent. In: Shah, N.S. and Donald, A.G. (eds.). Psychoneuroendocrine Dysfunction in Psychiatric and Neurological Illnesses: Influence of Psychopharmacological Agents, New York, Plenum Press (in press).

Kleinman, J.E., Reid, A., Lake, C.R. and Wyatt, R.J.: Studies of norepinephrine in schizophrenia. In: Ziegler, M.C. and Lake, C.R. (eds.). Norepinephrine, Baltimore, Williams and Wilkins (in press).

Jeste, D.V., Linnoila, M., Wagner, R.L. and Wyatt, R.J.: Serum neuroleptic levels and tardive dyskinesia. Psychopharmacology (in press).

Jeste, D.V. and Wyatt, R.J.: Neuroleptic-induced tardive dyskinesia. In: Akhtar, S. (ed.). New Psychiatric Syndrome, New York, Jason Aronson (in press).

Wyatt, R.J. and DeLisi, L.E.: Future research directions in the treatment of schizophrenia. Canadian Medical Journal (in press).

Weinberger, D.R. and Wyatt, R.J.: Enlarged cerebral ventricles in schizophrenia. Psychiatric Annals (in press).

Wyatt, R.J. and Weinberger, D.R.: Future directions for biological exploration of psychiatric disorders. In: Sullivan, J.L. (ed.). Principles of Psychiatric Medicine, Massachusetts, Butterworths (in press).

Karson, C.N., Jeste, D.V., LeWitt, P.A. and Wyatt, R.J.: A comparison of two iatrogenic dyskinesias. American Journal of Psychiatry (in press).

Liebowitz, M.R., Karoum, F., Quitkin, F.M., Davies, S.W., Stewart, J.W., McGrath, P.J., Harrison, W., Schwartz, D., Levitt, M., Linnoila, M., Wyatt, R.J., Palij, M. and Klein, D.F.: Biochemical effects of deprenyl. Psychopharmacology Bulletin (in press).

Morihsa, J.M., Duffy, F.H. and Wyatt, R.J.: Brain electrical activity mapping (BEAM) in schizophrenic patients. Archives of General Psychiatry (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01337-12 SMRA
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies on Drugs of Abuse		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Richard J. Wyatt, M.D., Chief, Adult Psychiatry Branch, NIMH		
COOPERATING UNITS (if any) National Institute on Alcohol Abuse and Alcoholism and George Washington University		
LAB/BRANCH Adult Psychiatry Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: <div style="text-align: center;">5</div>	PROFESSIONAL: <div style="text-align: center;">4</div>	OTHER: <div style="text-align: center;">1</div>
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input checked="" type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input checked="" type="checkbox"/> (b) Human tissues </div> <div> <input type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Studies of drugs of abuse have focused primarily on the effects of alcohol consumption. Studies have been performed to assess the effects of alcohol consumption on memory functions. This has been examined both clinically and through basic science studies examining hippocampal slices. Also, research is underway to investigate whether or not there is a relationship between MAO and alcohol consumption. An investigation into the drinking patterns of the elderly is being performed as has been a study of phencyclidine (PCP). </p>		

Projection Description

Drs. William J. Freed, T. Peter Bridge, Craig N. Karson, Joel Kleinman, John Morihisa, Farouk Karoum, H.E. Cannon-Spoor, Bruce H. Phelps, Richard Jed Wyatt and Mr. Paul Oliver from the Adult Psychiatry Branch, S. Hashtroudi from George Washington University, and Dr. Elizabeth Parker from NIAAA, performed studies on substances of abuse.

Objectives:

The objective of the Substance Abuse Program in the Adult Psychiatry Branch is to formulate and investigate hypotheses concerned with the nature and action of pharmacological agents that are either classified as or can become, through misuse, drugs of abuse. Through research examining the mechanisms of action of these substances, we hope to better understand their use and the effects of their abuse.

Alcohol

Memory

A major goal of research on alcohol and memory has been to identify the memory processes that are disrupted during intoxication. There is compelling evidence that failure to engage in elaborative processing is a critical source of alcohol-related memory impairment. Recent studies, however, suggest that certain forms of memory or certain memory processes are independent of semantic elaboration. These processes are relatively unimpaired in amnesic patients, including alcoholic Korsakoff patients, and might also be resistant to amnesia produced by acute alcohol intoxication. To pursue this possibility further, Dr. Hashtroudi and colleagues examined the differential vulnerability of memory processes to alcohol amnesia by drawing upon studies of amnesic syndrome.

Despite severe memory deficits in tasks requiring elaboration, amnesic patients show relatively normal retention in a variety of memory tasks. The early studies suggested that amnesics could acquire and retain motor skills and learn and perform maze problems. Researchers extended the domain of preserved memory processes to verbal materials. It was reported that amnesics did not differ from controls in identifying visually degraded words. Neither amnesic patients nor controls had been able to identify the degraded stimuli before being exposed to these words. After the study trials, however, the groups were indistinguishable in their identification performance. More recent evidence indicates that amnesics show considerable memory savings in reading inverted tests, and in interpreting homophones according to their recently biased meanings.

In accounting for these results, it has been suggested that, due to a deficit in attention, amnesic patients are less able to engage in elaborative and effortful processing. Thus, traditional memory tests such as recall, which require elaborative processing at input and respecification of context at output, are particularly susceptible to amnesia. On the other hand, memory tests that rely on automatic processing or judgement of perceptual fluency are resistant to amnesia. Prior experience with an item may be manifested in a variety of perceptual and classification tasks, but may not be tapped by traditional tasks. Identification of degraded words is an example of a task that is based on perceptual fluency or familiarity, but is not dependent on elaboration or interitem relationship.

In order to examine the differential vulnerability of memory processes to alcohol amnesia, we selected three different memory tasks: free recall, identification of visually degraded words, and recognition. Intoxicated and sober subjects studied the same list of words and participated in one of the three retention tests. The free recall test, which clearly requires elaborative processing, was included as a sensitive measure of acute alcohol amnesia. Impaired recall under alcohol should, therefore, provide a comparison for possibly preserved retention during intoxication.

The results demonstrate that there is considerable specificity in memory processes which are disrupted by alcohol. In agreement with previous research, alcohol impaired free recall of a list of words. In contrast, intoxicated subjects showed normal retention of the same list under certain conditions. In identification of degraded words, intoxicated subjects benefited to the same degree as sober controls from a single exposure to the item at study. Likewise, intoxicated subjects did not differ from sober subjects when the test conditions encouraged a recognition judgement based on perceptual information. We suggest that memory tasks that depend on elaboration or interitem integration are susceptible to alcohol amnesia, whereas tasks that rely on perceptual fluency or familiarity are resistant to disruption during intoxication.

Finally, the present results on alcohol amnesia support the distinction between different forms of memory, a distinction already suggested by studies of the amnesic syndrome and normal memory. Clearly, there is much to be learned about the conditions in which interdependence or independence between the different forms of memory might be observed. Nevertheless, the distinction necessitates a new approach for investigating the effect of alcohol on memory. This approach entails an examination of what is, as well as what is not, remembered during intoxication.

Neuronal Response in Hippocampal Slices

Recent studies have demonstrated that depending upon the sequence in which it is given, in both animals and humans, acute alcohol ingestion can either enhance or depress long-term retention of events. For example, in mice alcohol decreases long-term retention in a one-trial passive avoidance test given prior to learning, but enhances retention of the same test when given following acquisition. Similarly, when humans ingest a large dose of alcohol after studying to-be-remembered material, their long-term retention of this material is better than for non-drug controls. Drinking prior to presentation of the material decreases retention. Because of the surprising ability of alcohol to improve memory of predrinking events and the international importance of drinking-related problems, Mr. Oliver and his colleagues sought to learn if alcohol could produce similar enhancement-depressant effects in a relatively simple system.

Mr. Oliver and colleagues recently reported that it is possible to study long-term changes of neuronal excitability in hippocampal slices taken from pentylenetetrazol (PTZ) kindled animals. In slices taken from normal animals, a rhythmic but abnormal discharge called the interictal spike, requires a convulsant (penicillin) and a high concentration of potassium (7 millimoles or more). This enhanced response persists for at least 20 days after the kindling stimuli, suggesting an altered ionic mechanism might be involved. Therefore, kindling, a well-defined long-term change in neuronal excitability, provided a good model to determine whether alcohol might promote or inhibit long-term neuronal changes. To examine the response of neurons in hippocampal slices analogous to alcohol-induced changes in animal and human memory, Mr. Oliver examined hippocampal slices from rats injected with alcohol and pentylenetetrazol.

When alcohol was given after, but not before, three days of pentylenetetrazol administration, kindling equivalent to five days of pentylenetetrazol was produced. This effect appears to be independent of the known withdrawal effects of alcohol and lasts at least 14 days after alcohol and pentylenetetrazol administration has been terminated.

MAO and Alcohol

The literature contains reports from several laboratories linking abnormal catecholamine metabolism with the occurrence of alcoholism. Two major catecholamine--metabolizing enzymes, in particular, monoamine oxidase (MAO) and dopamine-beta-hydroxylase (DBH), have been studied intensively in attempts to correlate activity levels with alcoholism. A consensus of these studies seems to support the idea of lowered platelet MAO activity in this disease. The fundamental issue in all of these studies is whether altered MAO and DBH activities in alcoholic subjects are a result of heavy drinking and associated life-style or whether they represent pre-disposing genetic risk factors for the development of the disorder.

Drs. Bruce Phelps and Elizabeth Parker have tested this hypothesis by using the population survey method to define persons at high risk by age and family history. Platelet MAO activity was analyzed in a group of American male college students and in a second group of young Danish males. Also investigated, were the effects of acute alcohol intake on platelet MAO activity in a subgroup of the American student population.

Results showed that acute alcohol intake does not significantly affect platelet MAO activity. Low MAO activity, however, may be used to predict heavy, long-term alcohol use. Additionally, in conjunction with family history, low MAO activity may predict the manner in which alcohol is most likely to be abused; subjects with a positive family history of alcoholism and low MAO activity were found to drink significantly more per occasion while those with a negative family history and low MAO activity drink more frequently. Finally, it was shown we found that platelet MAO activity is higher in the Danish population and did not vary with family history of alcoholism. These results suggest culturally-dependent differences in the way MAO activity relates to alcohol use.

Alcohol and the Elderly

There is a growing sensitivity to what appears to be an increasing prevalence of alcohol consumption among the elderly, particularly around retirement. To begin to understand the alcoholic patterns of the elderly and what effects alcohol might have on cognitive functions, Drs. Peter Bridge and Elizabeth Parker are looking at a cross-sectional study of 200 subjects without evidence or history of psychiatric problems or alcohol or drug abuse. Particular attention is being directed to whether or not there are different alcohol consumption patterns associated with differential social drinking habits (binge versus moderate) with respect to advancing age and brain structure as shown on computerized tomography (CT) scans. Studies are examining neurochemical measurements and cognitive function.

Phencyclidine (PCP)

Widespread abuse of the drug phencyclidine (PCP) in recent years has caused an increasing number of hospitalizations due to severe and sometimes violent toxic reactions. These adverse reactions frequently resemble psychotic episodes, involving violence, agitation, and bizarre behavior, and can last for as long as several weeks. Frequently, therefore,

some form of pharmacological treatment is indicated. The preferred form of treatment is controversial, some authors recommending benzodiazepines while others prefer neuroleptics. In animals, a number of studies agree that at least some of the effects of PCP can be blocked by neuroleptics, but few compared various neuroleptics for their PCP-blocking efficacy.

Several studies have reported that both haloperidol and pimozide are effective blockers of various effects of PCP. One study found that pimozide is somewhat less effective than haloperidol in blocking PCP-induced rotational behavior. In addition, haloperidol has been found to be less effective than chlorpromazine and clozapine in blocking PCP-induced locomotor stimulation in mice. Another study in rats reported that butyrophenones, but not phenothiazines, antagonized some behavioral effects of PCP. These findings suggest that there may be substantial differences among neuroleptics in their ability to block the various effects of PCP. To test this hypothesis, Dr. William Freed and colleagues compared a variety of neuroleptics for their ability to block the stimulant effects of PCP in mice.

These data confirm a number of previous reports that behavioral effects of PCP in animals can be attenuated by neuroleptics. In general, the best PCP antagonists (methiothepin, fluphenazine, and trifluoperazine) are very potent clinically (on a mg/kg basis) in schizophrenia, while several of the less potent PCP antagonists (thioridazine, clozapine, molindone, and sulpiride) are also less potent clinically (on a mg/kg basis). Chlorpromazine, however, was very effective, even though it is not very potent clinically. And, pimozide and haloperidol were relatively ineffective despite their great clinical potency. Pimozide was essentially ineffective, and the blockade of PCP that was produced by haloperidol was not dose-dependent and was never complete. This suggests that some property of neuroleptics other than their primary therapeutic action is involved in their ability to antagonize PCP-induced stimulation in mice.

The neuroleptics are thought to act clinically, in the treatment of schizophrenia, by blocking central dopamine receptors. Many neuroleptics, however, have substantial anti-serotonergic activity as well. Dr. Freed and colleagues found that the ability of neuroleptics to block PCP was strongly correlated with their ability to block tryptamine-induced seizures, a presumed measure of antiserotonergic activity. PCP-blocking activity was also correlated with inhibition of spiroperidol binding in the frontal cortex and inhibition of LSD binding, both of which reflect antiserotonergic activity. In addition, significant correlations were also obtained with several dopaminergic measures, particularly inhibition of dopamine binding. These correlations were found only for certain measurements of dopaminergic and serotonergic properties. PCP does not, however, interact directly with dopamine binding sites. It may be that either a particular form of antiserotonergic or antidopaminergic activity is capable of blocking the effects of PCP or that a particular combination of antagonistic properties is effective.

Martin and colleagues (1979) reported that large doses of PCP induced a serotonergic syndrome, which could be blocked by serotonin antagonists. The serotonergic syndrome was followed by stereotyped behavior, which could be blocked by haloperidol. These findings reinforce the conclusions that the stimulation produced by PCP has both dopaminergic and serotonergic components. In a previous study Dr. Freed and colleagues found that PCP-induced stimulation could be blocked by yohimbine, an alpha-adrenergic antagonist. Another alpha-adrenergic antagonist, phentolamine, was ineffective. It has recently been reported, however, that yohimbine has both antiserotonergic and antidopaminergic properties, which

could explain its PCP-blocking activity. Methiothepin, the drug which we found to be most effective, is a potent antagonist of both serotonin and dopamine.

Despite the facts that the blockade of PCP produced by haloperidol was statistically significant, and that the ED_{50} was fairly small, haloperidol was conspicuous in that the blockade of PCP, measured as stimulation ratios, was not dose-dependent and never reached 100%. In an earlier study, the blocking effect of haloperidol was similar in terms of degree, but the effect did not reach statistical significance because fewer animals were tested. Thus, haloperidol appears to be only capable of partially blocking the effect of PCP. A significant effect of pimozide could not be demonstrated, even though large numbers of animals were tested. The highest dosage of pimozide, however, produced a nonsignificant partial decrease in stimulation ratios, which was similar in degree to that produced by haloperidol. Both pimozide and haloperidol have relatively little antiserotonergic activity. This suggests that haloperidol and pimozide may only have blocked the dopaminergic component of PCP-induced stimulation, while leaving the serotonergic component unaffected.

Consideration should therefore be given to the possibility that certain clinically-potent neuroleptics, the butyrophenones in particular, are relatively poor choices for the clinical treatment of PCP-induced toxicity. The present findings suggest that the most effective drugs for treatment of PCP-induced toxicity would be methiothepin, fluphenazine, thioridazine, and chlorpromazine, in that order. Other groups, however, have found certain behavioral effects of PCP to be blocked by drugs such as pimozide, serotonin antagonists, cholinergic drugs and reserpine. Therefore, the pharmacological profile of PCP may depend upon the behaviors or species of animals that are being used to measure its effects. In addition, alterations in PCP metabolism or side effects of neuroleptics, such as ataxia or cataleptic effects of butyrophenones could contribute to these effects. Consequently, it would be premature to recommend clinical therapies on the basis of any single type of experiment. If it can be shown that drugs such as methiothepine and fluphenazine are superior in blocking PCP across a range of behavioral tests, clinical experiments on that basis might be justifiable.

METHODS

Appropriate methods are employed and, where possible, studies are performed in a double-blind design. Also, whenever possible, sample sizes are large enough to allow for general conclusions.

Significance to Biomedical Research and the Program of the Institute

Our work in the drug abuse area has focused on the effects of alcohol consumption and the use of phencyclidine (PCP). The significance of performing research on these two substances becomes readily apparent when considering the pervasiveness of their misuse and the damage to the individual, family and society that their abuse brings.

Turning first to PCP, we find that PCP hydrochloride intoxication, especially among young people, has reached alarming and epidemic proportions. Abuse of PCP, often referred to as "angel dust" or "hog", frequently appears to result in violent behavior and mortality among its users. Consumption of PCP has a profound effect on mental status and is known to cause disorientation, psychosis, uncontrolled violent reactions and convulsions.

Because these violent reactions have been reported to last, in certain individuals, up to several weeks, pharmacological treatment is indicated. There is controversy, however, about a preferred form of treatment. Some clinicians recommend benzodiazepines, others recommend neuroleptics. Because of this treatment controversy, our findings relating to the differential effects of various neuroleptics on the amelioration of PCP-induced symptoms is of use to the medical community as well as the public, at large.

Our work with alcohol also is highly significant, particularly in light of the well-known statistic that there are in excess of ten million alcohol abusers in this country. And, even though we have rather precise estimates on the extent of alcohol abuse, we have little definitive information on the effects of alcohol on memory. Thus, our work, beginning of differentiate those mental functions most severely hindered by alcohol consumption, is of importance to both the general population and the scientific community.

Proposed Course

We plan to continue investigating the effects of various pharmacologic treatments on the symptoms of phencyclidine-induced psychosis, as well as the differential effects of alcohol consumption.

PUBLICATIONS

Karoum, F., Speciale, S.G., Chuang, L-W. and Wyatt, R.J.: Selective effects of phenylethylamine on central catecholamines: A comparative study of amphetamine. Journal of Pharmacology and Experimental Therapeutics 233: 432-439, 1982.

Stillman, R., DeRenzo, E., Wolkowitz, O., Allen R., Lehman, L. and Wyatt, R.J.: Development of differences in response latencies between right and left visual fields. Brain and Cognition (in press).

Hashtroudi, S., Parker, E., DeLisi, L.E. and Wyatt, R.J.: On elaboration and alcohol. Journal of Verbal Learning and Verbal Behavior (in press).

Karoum, F., Commissiong, J. and Wyatt, R.J.: Effects of morphine turnover in different functional regions of rat spinal cord. Biochemical Pharmacology (in press).

Chuang, L-W., Karoum, F. and Wyatt, R.J.: Different effects of behaviorally equipotent doses of amphetamine and methamphetamine by amphetamine. European Journal of Pharmacology (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01338-05 SMRA
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Studies of Aging		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Richard J. Wyatt, M.D., Chief, Adult Psychiatry Branch		
COOPERATING UNITS (if any) Karolinska Institute, Sweden; University of Colorado and National Institute of Alcohol Abuse and Alcoholism		
LAB/BRANCH Adult Psychiatry Branch		
SECTION		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 9	PROFESSIONAL: 7	OTHER: 2
CHECK APPROPRIATE BOX(ES) <input checked="" type="checkbox"/> (a) Human subjects <input checked="" type="checkbox"/> (b) Human tissues <input type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The Unit of Geriatric Psychiatry performs research into several aspects of the aging process. In the area of <u>Alzheimer's disease</u>, studies have been performed examining the roles played by <u>aluminum</u> and <u>fluoride</u>. Examining the differential effects of neuroleptic drugs on the elderly, <u>diagnostic criteria</u> for <u>tardive dyskinesia</u> have been developed as well as research being performed to measure <u>serum neuroleptic concentrations</u> in older patients. Also, studies of <u>cerebral ventricular size</u> have been performed. </p> <p> Another major thrust of the work of this unit is in the area of brain tissue grafting in <u>Parkinson's disease</u>. <u>Transplantation of fetal and adrenal medulla tissue</u> continues into the brains of parkinsonism model rats and monkeys. Histology techniques continue to be refined and inroads into questions of <u>immunocompatibility</u> are being pursued. And finally, in work also designed to eventually correct neural damage, research into <u>nerve repair</u> is progressing. </p>		

Project Description:

Drs. David Shore, Luis de Medinaceli, Craig Karson, Daniel Weinberger, Dilip Jeste, Peter Bridge, Allen Church, Bruce Phelps, H.E. Cannon-Spoor, Urmi Patel, Renaud de Beaurepaire, William Freed, and Richard Jed Wyatt, Elizabeth Parker from NIAAA, Ake Seiger and Lars Olson from Karolinska Institute and Barry Hoffer from University of Colorado performed studies on various aspects of the aging process.

Objectives:

The objectives of research performed in the Unit on Geriatric Psychiatry are to test existing hypotheses and create new hypotheses relating to the social, psychological, cognitive, physiological and affective changes that occur through the aging process. Further, it is our objective to perform research that illuminates the differences between normal aging and pathology, synthesizing work from specific disciplines as well as interdisciplinary efforts.

Senile Dementia

Aluminum Fluoride and Alzheimer's Disease

As we have explained in previous Annual Reports, the most common cause of "senility" in the elderly is a degenerative brain disease called Alzheimer's disease or Alzheimer-type senile dementia. This illness is characterized by a progressive decline in memory and intellect, and a deterioration of social, occupational and communication skills. It is estimated that three to five percent of the United States population over age 65 is afflicted with this disease. Many more Americans will be afflicted as the average age of our population continues to rise. There is, presently, no effective treatment for Alzheimer's disease.

In recent years, patients with Alzheimer's disease have been found to have accumulations of aluminum in the hippocampus and cortex of the brain. These accumulations are localized within the nuclei of those nerve cells showing the neurofibrillary degenerative changes typical of Alzheimer's disease. This degeneration is most commonly seen in areas of the brain that are associated, generally, with memory and higher mental functions.

In earlier work investigating the significance of the relationship between aluminum accumulations and Alzheimer's disease, we measured serum aluminum concentrations found in hospitalized patients of similar age and gender. The results tended to confirm that the increases in nerve cell nucleus aluminum reported in Alzheimer's disease are not the result of a generalized overload of this metal in biological fluids.

These findings, however, did not eliminate many of the questions concerning aluminum's role in the etiology and progression of Alzheimer's disease. If aluminum is a contributor to the degenerative brain changes in Alzheimer's disease, attempts to remove this metal from the body could have significant effects on the course of the illness. In this regard, the fluoride ion is of particular interest since elimination of aluminum by urine and feces is significantly increased by fluoride and aluminum retention in the body, reportedly, is decreased. A mutual reaction may occur between these ions in the body, resulting in the formation of an aluminum fluoride complex with the result that aluminum is not retained in the organism.

To examine this possibility further, Dr. Shore and colleagues initiated several preclinical studies investigating the aluminum-fluoride complexes. For several reasons, Dr. Shore focused on the use of fluoride to complex the aluminum which has already been absorbed, rather than trying to prevent the absorption of aluminum. One factor was interest in identifying patients early in the course of Alzheimer's disease and attempting to prevent the further progression of dementia. Earlier work showed that such patients do not have elevated concentrations of aluminum in blood or cerebrospinal fluid. Since most foods contain only 1.6 to 30 mg Al/kg, and normally only small amounts (less than 5%) of aluminum are absorbed, we have been more concerned with the potential toxicity of that aluminum already present in Alzheimer's patients. Such patients may have a "vulnerability" to aluminum neurotoxicity on the basis of genetic, viral, or other inability to prevent aluminum from accumulating on DNA in neuronal nuclei. Results of the preclinical studies using small samples showed that fluoride treated animals tended to have lower aluminum concentrations in the brain. These studies are being replicated with larger numbers of animals.

Dr. Shore is, also, conducting a double-blind placebo-controlled study of the ability of fluoride to prevent the progression of early Alzheimer's in outpatients. The results of this study will be available in the next one to two years.

In related work, Drs. Shore and Wyatt have been examining the research design problems associated with testing anti-aluminum drugs in humans and developing study criteria.

Neuroleptics

Tardive Dyskinesia

Neuroleptics constitute our most effective form of treatment for schizophrenia. Long-term administration of the antipsychotic agents, however, is fraught with the risk of inducing complications such as tardive dyskinesia that may be potentially irreversible. This danger is much greater in the elderly than in the young. The risk of tardive dyskinesia is high in both types of elderly patient populations: patients who have been diagnosed as having schizophrenia in early adulthood and who have continued receiving neuroleptics through old age; and elderly persons who, for the first time, develop psychosis and who are then placed on neuroleptic treatment.

Tardive dyskinesia may be defined as a syndrome consisting of abnormal, stereotyped involuntary movements, usually of choreoathetoid type, affecting the mouth, face, limbs, and trunk, which occurs relatively late in the course of drug treatment. While tardive dyskinesia occurs following treatment with drugs other than neuroleptics, neuroleptics are by far the commonest cause of iatrogenic tardive dyskinesia. To refine the process involved in prescribing these drugs.

Drs. Jeste and Wyatt proposed the following set of diagnostic criteria:

A. Phenomenology

- 1) The abnormal movements are choreiform (i.e., nonrepetitive, rapid, jerky, quasi-purposive movements), or athetoid (i.e., continuous, slow, sinuous, purposeless movements), or rhythmic abnormal involuntary movements in certain areas of the body,

which are reduced by voluntary movements of the affected parts and increased by voluntary movements of the unaffected parts.

- 2) The abnormal movements are increased by stress, and reduced when the person is relaxed. They may be temporarily controlled by volitional effort. The movements are absent during sleep.
- 3) One or more of the following three areas are usually involved in tardive dyskinesia: tongue, jaw, and extremities. Isolated involvement of other body parts (in the absence of dyskinesias of tongue, jaw, or extremity movement) is rare.
- 4) Tremors, acute dystonias, myoclonus, mannerisms, and compulsions are not a part of the dyskinesia syndrome. Some of these may, however, coexist with dyskinesias.

B. History

- 1) The movement disorder should be present for at least three weeks before tardive dyskinesia is diagnosed. Although the symptom severity may vary, the dyskinesia should be present continually over that period. (Acute and withdrawal-emergent dyskinesias usually disappear within one to three weeks of stopping the drug treatment).
- 2) The patient should have a history of administration of neuroleptics (for neuroleptic-induced dyskinesia) for at least three continuous months.
- 3) The same movements should not have been present before treatment with neuroleptics. The dyskinesia should have appeared either while the patient was on neuroleptics or within a few weeks of drug withdrawal.

C. Treatment and Response

- 1) Antiparkinsonian agents have no effect or cause worsening of tardive dyskinesia.
- 2) Increasing the dose of a neuroleptic usually reduces severity of dyskinesia. Dose reduction or withdrawal of neuroleptics aggravates the symptoms, at least temporarily.
- 3) Some catecholaminergic agents such as L-dopa and amphetamine make tardive dyskinesia worse.

D. Differential Diagnosis

The mere presence of the following conditions does not necessarily exclude a diagnosis of tardive dyskinesia. It is necessary, however, to show that these other conditions are not primarily responsible for the patient's dyskinesia. Ill-fitting dentures, use of drugs such as L-dopa or amphetamine, and Huntington's chorea are among the major causes of movement disorders that may mimic neuroleptic-induced tardive dyskinesia. Other conditions in differential diagnosis include heavy metal intoxication, liver and kidney damage, parathyroid disorders and several other rare neurologic syndromes.

The conditions that figure prominently in the differential diagnosis of tardive dyskinesia among the middle-aged and the elderly are as follows: a) Denture or dental

problems are probably the most common cause of non-tardive dyskinesias in this age group. In patients with dental problems or ill-fitting dentures, proper dental treatment results in alleviation of the oral dyskinesia. Usually, dyskinesias of dental origin are mild. b) Disorders of the basal ganglia, such as demyelination (as in multiple sclerosis), degeneration (e.g., Alzheimer's disease), neoplasms, and vascular pathology (e.g., arteriosclerosis), may occasionally result in orofacial dyskinesias. c) Nonneuroleptic drugs, especially L-dopa, used in the treatment of Parkinson's disease may produce dyskinesias. d) Spontaneous persistent dyskinesias occurring in the absence of any known cause of such abnormal movements occur, but are rare. They are similar in appearance to the tardive dyskinesias, but are generally mild.

There is a general consensus among most clinicians and researchers that the prevalence of neuroleptic-induced tardive dyskinesias increases with aging. Researchers have reported that the mean age of patients with tardive dyskinesia was considerably higher than that of nondyskinetic patients. Most of the studies comparing the prevalence of tardive dyskinesia in patients under 40 with that in patients over 40 found that the older patients had 50% to 2200% higher prevalence as compared with younger patients. The overall weighted mean prevalence of tardive dyskinesia in patients over 40 was nearly three times that of patients under 40.

Although one study concluded that women had a linear increase with age in the prevalence of tardive dyskinesia, while the prevalence in men decreased after age 70, such a gender-difference has not been found consistently in other investigations.

It may be argued that the increased prevalence of tardive dyskinesia associated with aging may be an artifact of the greater amounts of neuroleptics received by the elderly. Yet, in our patient population, we found that the mean daily doses of neuroleptics given to patients over 50 were several times lower than those prescribed for younger patients. Similarly, the greater prevalence of tardive dyskinesia in the aged cannot be explained on the basis of longer history of neuroleptic treatment. A number of studies have reported a lack of a significant correlation between prevalence of tardive dyskinesia and length of neuroleptic treatment. Furthermore, at least two groups of investigators found that age at onset of neuroleptic treatment was one of the most significant variables for discriminating between dyskinetic and nondyskinetic patient groups. It, therefore, appears unlikely that the association between aging and tardive dyskinesia is secondary to neuroleptic-treatment-related variables. It is, of course, conceivable that the elderly may receive polypharmacy for their multiple physical ailments. Some of these drugs (e.g., anticholinergic and antihistamine agents) may predispose to dyskinesia. The overall evidence suggests, however, that any contribution of such drugs to the etiology of tardive dyskinesia is probably minimal. We can thus conclude that there is likely to be some direct relationship between aging and susceptibility to tardive dyskinesia.

Concerning the clinical implications, tardive dyskinesia is not only common in the elderly (with prevalence figures in excess of 40% for inpatients with a history of prolonged neuroleptic treatment), but also tends to be severe and persistent (the rate of persistence after neuroleptic withdrawal is greater than 50%). Although a number of therapeutic strategies have been tried in dyskinetic patients, there is as yet no satisfactory method for treating this disorder.

Use of neuroleptics in the elderly should be restricted to specific indications such as those outlined in the American Psychiatric Association Task Force on Tardive Dyskinesia. There is little justification for prescribing neuroleptics as sedatives. Even relatively short-

term administration of these drugs to geriatric patients carries a risk of producing persistent tardive dyskinesia. The following is a list of suggested guidelines for using neuroleptics in the elderly.

- 1) Neuroleptics should be prescribed only for well-justified indications, in smallest effective doses and for shortest possible periods. The dosage requirements for older patients are often much smaller than the standard doses for younger adults given in the Physician's Desk Reference. The available data do not support a recommendation for drug-free periods to prevent tardive dyskinesia.
- 2) The risk of tardive dyskinesia should be discussed with the patients and their families.
- 3) Both the need for neuroleptics and the fact of having discussed the risk of tardive dyskinesia with patients and families should be documented.
- 4) Patients should be examined for the presence of dyskinesia before, and at least once a month after, starting neuroleptics. The findings should be documented.
- 5) Anticholinergic and antihistamine drugs should not be prescribed unless specifically indicated.
- 6) At the first signs of tardive dyskinesia, neuroleptics should be discontinued, or at least, their doses should be reduced. If it is necessary to continue these drugs, the reasons for doing so should be documented and a consent should be obtained from the patient and his or her guardian. Preferably, the type of drug may be switched to a different one. Although most of the commonly used neuroleptics are similar in terms of the risk of tardive dyskinesia, individual drugs may be better or worse for given patients.

In a follow-up study of an earlier study done with middle-aged and elderly female populations, Dr. Jeste and his colleagues measured serum neuroleptic concentrations with a liquid chromatographic assay for thioridazine, and its active biotransformation products. This assay quantifies concentrations of thioridazine and its three active biotransformation products mesoridazine, sulforidazine, and nortioridazine. Each of these metabolites has been shown to have different affinities for dopaminergic, α -noradrenergic, and muscarinic acetylcholine receptors. It has been suggested that the affinities of neuroleptics for different receptors may be related to specific therapeutic and side effects of the drugs.

Dr. Jeste and co-workers sought answers to the following questions: Is the ratio of serum neuroleptic concentration (measured by the liquid chromatographic assay) to the daily dose of the drug (thioridazine and mesoridazine) different in TD and non-TD patients? Does this ratio remain stable in individual patients when they are followed up over a one-year period? Which, if any, of the thioridazine metabolites in the serum is higher or lower in TD patients? Are serum α -1-acid glycoprotein concentrations, which are presumed to indicate inflammatory activity, different in TD and non-TD groups?

Measurement of serum concentrations of neuroleptics has been a recent development. At present, the relative contributions of various patient- and treatment-related factors to the concentration of neuroleptics in serum are not known satisfactorily. Hence, for our study, we decided to select two groups of patients, with and without TD, that were comparable on a number of possibly relevant variables including age, gender, height, weight, primary psychiatric diagnosis, total length of past neuroleptic administration, and type of

current neuroleptic treatment. Furthermore, all these patients had been under the care of the same psychiatrist, so that any therapist-related biases regarding treatment (e.g., use of adjuvant medications) could be expected to affect the two groups to a similar extent. We also excluded patients with mild or questionable dyskinesia from either group.

Our findings can be summarized as follows: 1) The mean ratio of serum concentration to daily dose of neuroleptic was significantly higher in TD patients than in non-TD patients; 2) the ratios for individual patients who continued to receive neuroleptics remained stable over a one-year period; 3) serum sulforidazine levels seemed to be significantly elevated in the TD patients; 4) TD and non-TD groups did not differ from each other in serum α -1-acid glycoprotein concentration, suggesting that the differences in serum neuroleptic concentration were not an artifact of peripheral inflammatory activity.

Thus, our results are consistent with the possibility that abnormal metabolism of neuroleptics may be related to the pathophysiology of TD, at least in a subgroup of patients. We should stress that we did not study pharmacokinetics of neuroleptics in a population: We measured the ratio of serum concentration to daily dose of neuroleptics. Such a ratio is a less than ideal index for comparing drug metabolism in two groups when all the patients are not matched for the daily dosages, since the relationship between dose and serum concentration of neuroleptics is not necessarily linear. Under the given clinical circumstances, the ratio seemed to be the most practical, albeit indirect means of assessing drug metabolism, although it should not be construed as a substitute for acute pharmacokinetic studies. We may also add that the mean ratios of our five dose-matched patients from each group were similar to those of the three patients who were not dose-matched from the same group.

Computed Axial Tomography

Over the past five years there has been a proliferation of research studies of schizophrenic patients evaluated by computed tomography (CT). This landmark radiological technique has revealed that some schizophrenic patients have CT scan findings suggestive of cerebral atrophy. The findings include enlarged cerebral ventricles, dilated cortical fissures and sulci, and possibly, reduced radiodensity of the cerebral parenchyma. Although negative studies have appeared, the majority of the controlled investigations have confirmed these findings.

For a variety of reasons, most investigators have concentrated on schizophrenic patients in the third and fourth decades of life. In fact, only one study has included patients over sixty years of age. The rationale for selecting primarily young patients is that since signs of cerebral atrophy are uncommon in this age group, subtle atrophic changes will be more readily appreciated. In elderly populations where CT findings consistent with cerebral atrophy are common, it would be more difficult to differentiate subtle pathology, possibly related to the schizophrenic illness, from the non-specific concomitants of normal aging.

Now that an association between CT findings suggestive of cerebral atrophy and schizophrenia has been demonstrated, a study of elderly schizophrenic patients offers some potentially novel insights and may help answer lingering questions about the role of psychiatric treatment in the etiology of cerebral atrophy. Dr. Weinberger and colleagues were interested specifically in three questions: 1) Are the CT findings described in young schizophrenic patient observable in elderly patients who have been ill for many years? 2) What impact does the aging process have on these CT findings? 3) Is cerebral atrophy caused by many years of psychiatric treatment, especially institutional and somatic?

The first question addressed was whether the same CT findings observed in young schizophrenic patients are seen in older patients as well. It was found that the group means were significantly different, a finding consistent with other studies. There was, however, considerable overlap between the groups. In each age decade, the schizophrenic patients had larger mean ventricular size. Furthermore, advancing age was associated with larger ventricles in both patients and controls.

For the cortical atrophy measures, the difference between patients and controls is less obvious. Although there was a trend for dilated cortical sulci in the schizophrenic patients, this did not reach statistical significance.

Turning to the question of the effects of aging, the cerebrospinal fluid-filled spaces of the brain increase in size with age. This has been demonstrated in many CT studies of normal aging, and the results of this study are consistent with these previous reports.

In light of their extreme duration of institutionalization and drug treatment, the elderly patients in this study are an ideal group for observing the cerebral toxicity of drug and institutional treatments. Do these forms of therapy cause cerebral atrophy in the form of enlarged ventricles and dilated fissures and sulci? In order to demonstrate this, the effect of age must be isolated.

The most simple way to do this is to add the normal aging effect (i.e., the mean VBR of elderly controls minus the mean VBR of the young controls) to the mean VBR of the young schizophrenics. The result should reflect an approximation of the inherent schizophrenic VBR plus the additive age effect of 36 years. If this is considerably less than the VBR of the elderly schizophrenic patients in the present study, then secondary causes of enlarged VBR must be considered (e.g., drug and institutional therapy).

Performing the computation, we found that the predicted VBR was 12.9, only 11 percent less than the actual VBR. This is probably within the expected error of the method. It appears, therefore, that the effects of prolonged institutionalization and drug treatment on ventricular size are minimal. The finding that elderly schizophrenic patients have much larger ventricles than do their younger counterparts is consistent with the effects of normal aging. In fact, relative to their control population, the older patients have less deviant VBR's than do the younger patients relative to their controls.

The effect of treatment on cortical structures is more difficult to assess. Since the difference between patients and controls disappears in the elderly, it seems unlikely that psychiatric treatment is a cause of cortical atrophy.

The first conclusion is that cerebral atrophy, seen on a CT scan as enlarged ventricles and less frequently as dilated cortical fissures and sulci, exists in elderly chronic schizophrenic patients in a more extreme form than can be explained solely on the basis of advanced age. In other words, the findings described in young schizophrenic patients have been confirmed in the present study. One potential problem with this conclusion involves the control group. If it were not truly representative of elderly individuals then the results might be spurious. A review of other studies of ventricular size in healthy elderly subjects indicates that considerable variability of normal ventricular size exists across elderly samples.

The second conclusion suggested is that at least for ventricular enlargement, the age factor is additive to the schizophrenia factor. Aging does not appear to influence

ventricular size in a qualitatively distinct manner for either schizophrenic patients or normal controls. The additive nature of the aging factor may have clinical implications. Whereas ventricular enlargement is clinically mild in younger patients, when combined with the effect of aging it may become quite marked. Patients with this degree of atrophy may have less cerebral compensatory capacity. Further study is necessary to clarify the implications of this finding.

The most obvious need for future work in this area is to confirm the preliminary findings described. This will be necessary before additional projects can be pursued. It cannot be overemphasized that the findings reported here would be confirmed most conclusively in a longitudinal study. The present study was a cohort comparison that has limitations for investigating long-term effects. Questions about the etiology and pathogenic relevance of ventricular enlargement are probably best considered in younger patients where the aging factor is less of a complication.

It seems to us that this study raises certain issues that are particularly germane to the field of schizophrenia in later life. Foremost among them is the question of how schizophrenic dementia relates to cerebral atrophy. It has long been known that many elderly schizophrenic patients achieve a "burned out" state that in many ways is indistinguishable from dementia. The only clinical difference between it and presenile dementia of the Alzheimer type is its slower progression. One could hypothesize that large ventricles in early life predispose a schizophrenic patient to reaching the burned out state in later life. Perhaps the effects of aging, which have only minor implications for cognitive function in most individuals, are especially problematic in cases with pre-existing atrophy. This could be tested by evaluating cognitive performance in elderly chronic schizophrenic patients grouped according to ventricular size. Grouping patients by ventricular size is a research strategy that has been productive in research with younger patients. It may reduce the problems of heterogeneity common to samples of schizophrenic patients. This strategy should prove useful in research with elderly populations as well.

Nerve Repair

It is considered axiomatic that complete severance of a peripheral nerve results in an absence of conduction across the gap, even if the nerve is repaired. Regardless of the method of repair, impulses initiated in the proximal stump of a severed nerve have not been recorded in the distal stump prior to restoration of the continuity of the fibers by regeneration. The purpose of our work is to determine if, and under what conditions, conduction can be obtained following reconnection of the stumps of a freshly transected peripheral nerve.

Recovery from peripheral nerve injury can be studied by a wide variety of techniques. These include nerve and muscle electrophysiology, clinical or functional tests, the pinch test, measurements of axonal transport, and histology. The most important criterion, however, is the degree of functional recovery. Although easily assessed in man, in animals functional recovery has been difficult to measure.

Evaluation of sensory function is imprecise because of overlapping innervation and because measurements of sensory function are usually indirect. For this reason, recovery of motor function is a better criterion, even though it returns somewhat more slowly than sensory function. It has been shown that the loss of toe spreading is a very reliable measurement of peroneal nerve injury in rabbits, but this sign is difficult to quantify, either

by estimation or direct measurement. Other signs that have been used include foot-drop estimation and the "flick test" although the difficulties in quantification remain.

We suspected that the study of footprints, which are sometimes used to evaluate neurological deficits in animals would provide a more precise method. The study performed by Dr. de Medinaceli and colleagues describes an index based on measurements of the footprints of walking rats, which provides a reliable and easily quantifiable method of evaluating the functional condition of the sciatic nerve. Results of this method, the sciatic functional index (SFI) provide a very precise and reliable measure of rat sciatic nerve condition and is quickly and easily applied to large populations of animals. We are now doing that and expect to have the data from these experiments analyzed within the coming year.

Parkinson's Disease

Brain Grafts

Our work on tissue brain grafts has continued to expand since last year's Annual Report. As has been explained previously, rats with unilateral lesions of substantia nigra (SN) pars compacta, the area of the brain containing most dopamine-containing neurons, are a widely recognized animal model of Parkinson's disease. When given dopamine agonists such as apomorphine, these rats rotate in a direction contralateral to the lesion, presumably because of the development of supersensitive dopamine receptors in the striatum ipsilateral to the lesion. When grafts of embryonic SN are placed in the lateral ventricle, or into a transplant cavity adjacent to the striatum in animals with SN lesions, this rotational behavior has been shown to decrease. Histochemical examinations have shown that axons from the grafts have grown into the striatum, and biochemical measurements indicate that dopamine concentrations are increased in areas of the striatum adjacent to the SN grafts.

In our original work, cited in previous reports, we have transplanted fetal rat tissue into the denervated substantia nigra of adult rats. We have been watching the progress of these rats to assess the prolonged survival and success of the transplants.

The rats were assessed behaviorally at six months and histologically at nine months. Behaviorally, the animals continued to show significantly decreased rotation. Histologically, the grafts were studied for dopamine content and were found to be producing large amounts and reinnervating the host caudate nuclei. Interestingly, at eight to nine months, the transplants, unlike the rest of the animal's brains, showed no signs of aging.

One obvious problem with this technique, however, both for basic research and possible clinical applications, is the requirement for fetal central nervous donor tissue. To circumvent this problem, we sought other cells to substitute for the fetal tissue. We found that the adrenal medulla contained some cells with similarities to some substantia nigra cells. There are several reasons for considering the adrenal medulla as a potential replacement for fetal SN grafts. First, the normal adrenal medulla produces dopamine as an intermediary in the synthesis of adrenaline. Second, adrenal chromaffin cells, which are normally rounded in shape, become angular and develop processes when grown as grafts in the anterior eye chamber or when grown in culture in the absence of corticosteroids. Finally, processes originating from intraocular adrenal medulla grafts can innervate intraocular grafts of cerebral cortex.

The observations suggest that adrenal medulla grafts can reduce lesion-induced rotational behavior, even though there was no clear evidence that grafts actually reingranted

and produced fine fibers, these fibers were found almost entirely within the grafts, and rarely penetrated into the caudate nucleus. This suggests that catecholamines or other substances diffused from the grafts to receptor sites in the caudate nucleus in sufficient quantity to reduce caudate dopaminergic supersensitivity and consequently, apomorphine rotation.

To further substantiate the results of our grafting research, we have been seeking more sophisticated means of histologic examination. It is a relatively simple matter to locate and identify several tissue fragments transplanted within the central nervous system. Intraventricular grafts stand out as tissue islands within a fluid space, and even intraparenchymal grafts generally display sharp borders and histological appearance distinct from the surrounding host brain.

Fluorescence histochemical studies of the two types of graft tissue, using a glyoxylic acid technique revealed that both the SN and adrenal medulla grafts survived and contained specific fluorescence indicating the presence of catecholamines. The pattern and distribution, however, of catecholamine fluorescence in the two types of tissue differed markedly. SN graft tissue was moderately fluorescent, containing cells resembling those in the SN grafts pars compacta. The bulk of the catecholamine fluorescence associated with SN grafts was usually, however, found not in the graft per se, but as a fiber reinnervation of nearby regions of the host striatum.

Adrenal medulla grafts, in contrast, contained numerous brilliantly-fluorescent cells. These cells were similar in many cases to normal adrenal chromaffin cells, but a substantial proportion of these cells had either become elongated or developed short processes. These fibers did not, however, reinnervate the host brain. Most adrenal medulla grafts contained regions of very tightly packed catecholamine-containing cells with intense specific fluorescence, suggesting the presence of high catecholamine concentrations. Around these areas, secretion and diffusion of catecholamines could be seen as a fluorescent "halo" or cloud in the adjoining tissues.

Thus both SN and adrenal medulla grafts in the lateral ventricles decrease lesion-rotational behavior, but by two different mechanisms. SN grafts contain spontaneously-active dopaminergic neurons and reinnervate the host striatum. These grafts, therefore, decrease rotational behavior apparently by releasing dopamine from terminals into the denervated striatum of the host brain. In contrast, adrenal chromaffin cell grafts release catecholamines directly from their cell bodies: the catecholamines apparently reach receptor sites in the denervated striatum by passive diffusion. These techniques therefore provide two contrasting methods of decreasing the effects of SN lesions by tissue transplantation, one which relies on reinnervation and one which relies on secretion and diffusion.

In addition to the continuing studies with rats, Dr. Freed and his team are testing the effects of tissue brain grafts in rehesus monkeys. The findings will be reported in future reports.

Methods

Appropriate methods are employed and, where possible, studies are performed in a double-blind design. Also, whenever possible, sample sizes are large enough to allow for general conclusions.

Significance to Biomedical Research and the Program of the Institute

The significance of our research into the problems of the aging process are best seen in the areas of Parkinson's disease, senile dementia of the Alzheimer's type, and tardive dyskinesia, nerve repair and cerebral atrophy. In light of the shift in our nation's demographics towards an older population, increased understanding of these disease processes, neurological findings, and new methods of treatment are critically needed.

For example, Parkinson's disease, manifested primarily by abnormalities of movement and posture, is characterized by dopaminergic neuronal loss and gliosis in the brain. Current therapeutic approaches to Parkinson's disease involve administration of the drug L-dopa, a precursor of dopamine and dopamine-like agents. Despite some dramatic improvements, such therapeutic regimens are frequently not completely effective, or are associated with severe side effects. Many of these difficulties may result from, among other possibilities, the absence of the physiological mechanisms which normally regulate neurotransmitter release from dopaminergic terminals. Our work grafting dopamine-producing cells into the brains of Parkinson model animals attempts to circumvent this problem by developing a technique that would allow a previously damaged brain to begin reproducing the necessary dopamine. In developing this line of investigation intensive study has been generated internationally, leading to the first grafting operation in a human subject in Sweden.

Our examinations of the possible mechanisms involved in Alzheimer's disease are equally significant to the scientific community and the general population. Dementia is the major neuropsychiatric disorder of old age. According to figures issued by the National Center for Health Statistics, organic brain syndromes afflict 58% of the more than one million Americans in nursing homes. Many senile dementia patients are housed in other chronic-care facilities such as state mental hospitals and Veterans Administration hospitals. More than half of the patients over age 65 in state and county mental hospitals also carry the diagnosis of chronic organic brain syndrome or senile dementia.

Senile dementia of the Alzheimer's type can be defined as progressive, age-related, chronic cognitive dysfunction. A number of hypotheses have been promulgated to explain the origins of the disease. One that has attracted much attention in recent years postulates an increased amount of aluminum in the brains of Alzheimer's patients. Our work into the possible role of aluminum, as well as our investigations of various potential drug treatments, is timely and needed research.

Our work into the prevention and treatment of tardive dyskinesia, also, is of vital importance to both the medical and lay populations. Tardive dyskinesia is the most serious complication of long-term neuroleptic therapy. What was initially thought to be a rare clinical curiosity has become a significant public health hazard.

Typically, tardive dyskinesia occurs after years of neuroleptic administration. The syndrome consists of abnormal involuntary movements of the mouth and face, extremities, and trunk. The pathophysiology of tardive dyskinesia is not precisely understood and there is no satisfactory treatment. Our award winning investigations into this disorder have generated both significant findings as well as new research directions in many other laboratories.

Proposed Course

We plan to continue our work into the prevention and treatment of Parkinson's disease, senile dementia of the Alzheimer's type, tardive dyskinesia, nerve repair and cerebral

atrophy in the elderly. In our Parkinson's disease research our grafting work is expanding to include dopamine-producing tissue grafts into the brains of monkeys. In our Alzheimer's disease research we will be examining further the effects of sodium fluoride on the amelioration of the disease's symptoms as well as experimenting with potential pharmacological treatments. In our tardive dyskinesia work, we will be examining the potential of various enzymes as biological markers to determine which patients are at highest risk to develop the disorder. Our nerve repair research is continuing with refined means of quantification. Finally our CT work in elderly may, some day, help us subtype the schizophrenias. By determining those most at risk, we should then be better able to develop treatment modalities that both control the patient's psychosis while reducing the risk that the patient will develop tardive dyskinesia.

PUBLICATIONS

de Medinaceli, L., Freed, W.J. and Wyatt, R.J.: An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks. Experimental Neurology 77:634-643, 1982.

Wyatt, R.J., Freed, W.J., Hoffer, B.J. and Olson, L.: A novel method for local administration of neurally active agents. In: Fann, F. and Eisdorfer, C. (eds.). Clinical Psychopharmacology and Aging, New York, Springer, 1982, pp. 70-74.

Wuerthele, S.E., Yasuda, R.P., Freed, W.J. and Hoffer, B.J.: The effect of local application of homocysteine on neuronal activity in the central nervous system of the rat. Life Science 31:2683-2691, 1982.

Freed, W.J.: N,N-Dimethylglycine, betaine, and seizures. New England Journal of Medicine (in press).

Freed, W.J., Hoffer, B.J., Olson, L. and Wyatt, R.J.: Transplantation by brain tissue to restore the functional capacity of the damaged nigrostriatal system. In: Sladek, J. and Gash, D. (eds.). Neural Transplants: Development and Function, Plenum Publishing Co., New York, 1983.

Freed, W.J.: Functional brain tissue transplantation: Reversal of lesion-induced rotation by intraventricular substantia nigra and adrenal medulla grafts with a note on intracranial retinal grafts. A.E. Bennett Neuropsychiatric Research Foundation Award Paper for 1983 and Biological Psychiatry (in press).

Freed, W.J.: Brain tissue transplantation applied to the nigrostriatal dopamine system. International Medicine (in press).

de Medinaceli, L. and Freed, W.J.: Peripheral nerve reconnection: (I) Distributed mechanical support: Immediate histological consequences. Experimental Neurology (in press).

Wyatt, R.J. and Freed, W.J.: Grafting dopamine-containing cells into the striatal region of substantia nigra-lesioned rats. In: Proceedings of the World Health Organization Study Group of Neuroplasticity and Repair in the Central Nervous System (in press).

Wyatt, R.J. and Freed, W.J.: Progress in neurografting as a treatment for degenerative brain disease: The Parkinson's model. In: Regelson, W. (ed.). Intervention in the Aging Process, New York, Alan R. Liss, Inc. (in press).

Jeste, D.V. and Wyatt, R.J.: Aging and tardive dyskinesia. In: Miller, N.E., et al. (eds.). Schizophrenia, Paranoid and Schizophreniform Disorders in Later Life, NIMH Publications (in press).

de Medinaceli, L., Wyatt, R.J. and Freed, W.J.: Conditions which promote the return of function following complete transection of the sciatic nerve in rats. Journal of Neurosurgery (in press).

Weinberger, D.R., Jeste, D.V. and Wyatt, R.J.: Cerebral atrophy in elderly schizophrenic patients: Effects of aging and of long-term institutionalization and neuroleptic therapy. In: Miller, N.E., et al. (eds.). Schizophrenia, Paranoia and Schizophreniform Disorders in Later Life, NIMH Publications (in press).

Jeste, D.V., Jeste, S.D. and Wyatt, R.J.: Reversible tardive dyskinesia: Implications for therapeutic strategy and prevention of tardive dyskinesia. In: Bannet, J. and Belmaker, R. (eds.). New Research in Tardive Dyskinesia, New York, Plenum Press, (in press).

Wyatt, R.J. and Freed, W.J.: Central nervous system grafting. In: Wilkins, R.H. (ed.). Neurosurgery, New York, McGraw Hill, (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01500-II SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Indolealkylamines and neuronal function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. Economou-Hadjiconstantinou Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Biochemical Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our objective is to determine the role of <u>serotonin</u> in <u>spinal cord</u> and to see whether serotonin plays a role in <u>peripheral nerve function</u> .		

Proposed Course:

This work was done in collaboration with P. Panula, Visiting Fellow; Z. Lackovic, Visiting Associate; P.E. Potter, Guest Worker and N.H. Neff, Chief of the Section on Biochemical Pharmacology, SMRP, NIMH.

This work has been terminated and has been submitted for publication.

Publication:

Hadjiconstantinou, M., Potter, P.E. and Neff, N.H.: Transsynaptic modulation via muscarinic receptors of serotonin-containing SIF cells of superior cervical ganglion. J. Neurosci. 2: 1836-1839, 1982.

NOTICE OF INTRAMURAL RESEARCH PROJECT

Z01 MH 01503-09 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Pharmacological studies of acetylcholine turnover: control of cholinergic pathways

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

D.L. Cheney Chief, Section of Molecular Pharmacodynamics SMRP NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

0.7

PROFESSIONAL:

0.4

OTHER:

0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Although increases in plasma choline cause some increase in brain tissue choline, there is no increase in acetylcholine levels or in acetylcholine turnover rate in any of the brain areas studied. Indeed, increased plasma choline reduced the turnover rate of acetylcholine in the hippocampus demonstrating that increasing the availability of choline does not increase the rate of acetylcholine synthesis. Subcutaneously injected apomorphine appears to have a biphasic effect on the turnover rate of acetylcholine. Lower doses appear to reduce the turnover rate of acetylcholine in the striatum whereas at higher doses the turnover rate returns to normal.

Proposed Course:

This project was done in collaboration with J. Wroblewski, Visiting Fellow and Mr. H. Thompson, Psychologist, SMRP, NIMH.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01505-10 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Neurotransmitter dynamics: Chlordecone		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) O. Gandolfi Guest Worker SMRP NIMH		
COOPERATING UNITS (if any)		
None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Pharmacodynamics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.1	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Adult male rats receiving a single i.p. injection of <u>kepone</u> (80 mg/kg) exhibit tremors within a few hours after the injection. Since <u>kepone-elicited tremors</u> are relieved by injections of muscarinic receptor blockers, we measured <u>acetylcholine turnover</u> in various brain structures. We failed to detect evidence for an involvement of cholinergic presynaptic mechanisms in <u>kepone toxicity</u>. <u>Kepone</u> inhibits the turnover rate of <u>GABA</u> in striatum. Moreover, we found that <u>kepone</u> down-regulated <u>5HT₁ receptors</u> and increased the turnover of serotonin in hippocampus and striatum. These results suggest the possibility that <u>kepone</u> decreases GABAergic tone indirectly by an increase of serotonergic firing thereby increase cholinergic tone in striatum, causing tremors.</p>		

Project Description:

This project was done in collaboration with M. Barbaccia, Visiting Fellow and D.L. Cheney, Chief of the Section on Molecular Pharmacodynamics, SMRP, NIMH.

The objective of this study was to study neurotransmitter mechanisms which cause chlordecone toxicity.

Chlordecone (decachlorotetracyclodeconone) is a polycyclic chlorinated compound also known by the commercial name Kepone. Previous studies indicate that the neuronal system, the reproductive system, and the liver are major targets of Kepone toxicity. It has been found to cause neurotoxicity in man; these signs include tremors, headaches, abnormal elevation of cerebrospinal fluid pressure, mental symptoms, and visual disturbances.

Rats receiving the chlorinated insecticide, chlordecone (80 mg/kg i.p.), exhibit hyperexcitability, exaggerated startle response and tremors within a few hrs after the injection. The tremors persist several days and indirectly are responsible for the marked reduction in weight of the rats surviving for 7 to 10 days. Since the chlordecone-elicited tremors are relieved by injections of muscarinic receptor blockers, acetylcholine has been implicated indirectly in the mechanism of chlordecone toxicity. To check acetylcholine's participation in the toxicity of chlordecone, we measured acetylcholine steady state and turnover in various brain structures. We failed to detect evidence for an involvement of cholinergic presynaptic mechanisms in the chlordecone toxicity. Since in several structures GABA and acetylcholine may impinge on the same neuron exerting opposite effects (ACh excites while GABA inhibits firing) we have studied whether the GABA turnover changes in structures that could be involved in the genesis of tremor. We measured GABA turnover in hippocampus, striatum, brainstem and cerebellum and found that chlordecone inhibits the turnover rate of GABA in striatum but not in the other structures we have studied. Hence the inhibition of chlordecone-induced tremors by muscarinic receptor blocker could be studied by assuming that the following model operates in striatum.

Chlordecone would decrease GABAergic activity by an indirect action and thereby increase cholinergic tone, causing tremors. That an increase in cholinergic tone in striatum causes tremors was demonstrated with the pharmacological profile of oxytremorin. However our experiments fail to show what is the initial site of action of chlordecone. Since it is known that chlordecone causes an increase in serotonin turnover, we tested whether serotonin recognition sites were modified as a result of chlordecone injection. We found that there is a down regulation of serotonin (5HT₁) receptors with no change in 5HT₂ receptors. In conclusion our results favor the possibility that the tremors by chlordecone, are caused by a decrease of GABAergic tone in striatum elicited by an increase of 5HT₁ receptor activation. We do not know whether this serotonergic response is primary or secondary to another yet unknown mechanism.

The neurotoxicity of Kepone include tremors, headaches, abnormal elevation of cerebrospinal fluid pressure, mental symptoms and disturbance of vision. Hyperexcitability has been reported to occur in both man and mouse. The present study was performed to determine whether such neurotoxic effect could be mediated by effects on neurotransmission.

The course of the present research is in progress aiming to clarify if the serotonergic effect of the insecticide is primary or secondary to another yet unknown mechanism.

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZOI MH 01506-09 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Narcotic analgesics and the regulation of catecholamine neurons

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

Y. Gutman

Visiting Scientist

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Neuroendocrinology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

1.0

PROFESSIONAL:

1.0

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The role of opiate receptors located on catecholaminergic cells was studied using primary cultures of adrenal chromaffin cells. These cells contain opiate receptors in measurable amounts. Stimulation of these receptors with agonists decreases the release of catecholamines elicited by nicotine. This effect is stereospecific and is reverted by naloxone and diprenorphine. One of the most potent opiate peptides is met-enkephalin-Arg⁶-Phe. This peptide is present in the splanchnic nerve with acetylcholine. Upon release it may function as a cotransmitter for acetylcholine modulating the number of acetylcholine receptors available.

Project Description:

This project was done in collaboration with L. Saiani, Visiting Fellow; A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Enkephalin-like peptides coexist in association with other neurotransmitters in many axons, including the splanchnic (cholinergic) nerve endings and the chromaffin cells of the bovine adrenal medulla. They are released from the latter in association with catecholamines. It is hypothesized that these enkephalin-like peptides act as cotransmitters or neuromodulators of the primary transmitter. Our objective was to study the modulatory role of opiates on the acetylcholine (ACh)-induced catecholamine release from cultured bovine adrenal medulla cells. These cells possess high affinity, stereospecific opiate binding sites; the addition of opiate receptor agonists to these cells inhibits the ACh-induced release of catecholamines, but not the release elicited by KCl or Ca^{++} ionophores. Thus the primary culture of adrenal chromaffin cells is an ideal model where to study the molecular mechanisms by which the narcotic analgesics control the function of catecholamine containing cells.

Results

For this study we have used various agonists with different affinities for μ , δ , σ and κ receptors and we have compared the same compounds for their ability to bind to adrenal membranes and for their potency to inhibit the ACh-induced release of catecholamines from chromaffin cells. Etorphine, β -endorphin, met-enk[Arg⁶-Phe⁷] and the synthetic peptide D-Ala⁴, Me Phe⁴, [Met(O⁵-ol]-enkephalin inhibited the acetylcholine-induced release of catecholamines with an IC_{30} varying from 10^{-7} to 1×10^{-6} M. The effect was stereospecific because levorphanol ($\text{IC}_{30} = 7.5 \times 10^{-7}$ M) was approximately 2 orders of magnitude more potent than dextrorphan. Morphine (μ receptor agonist), D-Ala²-D[Leu⁵]-enkephalin (δ receptor agonist), ethylketazocine (κ receptor agonist) and N-allylnormetazocine (σ receptor agonist) were at least 100-1000 times less potent than etorphine. Diprenorphine ($\text{IC}_{50} 5 \times 10^{-7}$ M) and naloxone ($\text{IC}_{50} 10^{-6}$ M) antagonized the effect of etorphine. High affinity, saturable and stereospecific binding sites for ³H-etorphine, ³H-dihydromorphine, ³H-[D-Ala²-D-Leu⁵]-enkephalin, ³H-ethylketazocine and ³H-N-allylnormetazocine, ³H-diprenorphine and ³H-naloxone were detected in chromaffin cell membranes and in membranes obtained from adrenal medulla homogenates. However the number of binding sites for ³H-etorphine and ³H-diprenorphine was 10 to 70 times higher than the number of sites measured with the other ³H ligands. The rank order of potency of these compounds for the displacement of ³H-etorphine binding correlates ($r=0.96$) with the rank order of potency of the same compounds for the inhibition of ACh-induced catecholamine release. These data suggest that a stereoselective opiate receptor (different from the classical μ , δ , κ or σ receptor) with high affinity for etorphine, diprenorphine, β -endorphin and met-enk[Arg⁶, Phe⁷] modulates the function of the nicotinic receptor in adrenal chromaffin cells. Adrenal cells contain receptors for muscimol and benzodiazepines. Their function in catecholamine and opiate peptide release is now being investigated.

Proposed Course

We intend to study the molecular mechanisms by which the stimulation of opiate receptors produce a decrease of acetylcholine-induced release of catecholamine from adrenal medulla cells.

Conclusions

The opiate receptors are present in membranes of adrenal chromaffin cells. Activation of these receptors causes a non-competitive inhibition of the release of catecholamines elicited by the stimulation of nicotinic receptors. From our data, it can be inferred that when the met-enkephalin-like material in terminals of splanchnic nerve is released, it modulates the release of catecholamines induced by the stimulation of nicotinic receptors elicited by the concomitant neurally mediated release of acetylcholine. Alternative modulation of adrenal nicotinic receptor can be achieved by opiate-like peptides from blood (i.e. β -endorphin) or from the adrenal cells themselves. The adrenal medulla contains several types of opioid-like peptides; they include met- and leu-enkephalin-like peptides, dynorphin 1-13, peptide E, met-enk Arg⁶, Phe⁷, BAM 12P, BAM 20P and BAM 22P. Interestingly met-enk[Arg⁶, Phe⁷], a peptide present in high concentrations in adrenal medulla, and in the splanchnic nerve terminals (Panula, of this lab) is one of the most potent opiates tested. There is discussion whether this peptide acts by itself or after being converted to met-enk. Our data provide clear evidence that met-enk[Arg⁶, Phe⁷] is an opiate agonist on its own right because met-enkephalin and DADLE (a stable analogue of enkephalin) are two to three orders of magnitude less potent than the heptapeptide. We propose that met-enk-arg-phe is the putative endogenous opiate agonist which modulates the function of nicotinic receptors in adrenal medulla.

The interrelations between the opiate agonists and the nicotine-induced catecholamine secretion are relevant to the missions of the NIMH in many ways. The present study establishes a model to investigate how opiate receptors stimulation interact in the regulation of the activity of postsynaptic cells. On a more general ground these studies may help to learn the biological principle that regulates the functional interaction of a primary transmitter and coexisting neuropeptides in nerve axon terminal.

Publications:

Saiani, L., and Guidotti, A.: Opiate receptor mediated inhibition of catecholamine release in primary cultures of bovine adrenal chromaffin cells. J. Neurochem. 39: 1669-1675, 1982.

Costa, E., Guidotti, A., Hanbauer, I., and Saiani, L.: Modulation of chromaffin cells nicotinic receptor function by opiate recognition sites highly selective for met-enkephalin - [Arg⁶-Phe⁷]. Fed. Proc., in press, 1983.

Guidotti, A., Saiani, L., Wise, B.C., and Costa, E.: Cotransmitters; pharmacological implications. J. Neuronal Trans. Suppl. 18: 213-225, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 MH 01508-13 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Modulation of brain cholinergic function by neuromodulators and neuroactive compounds

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

W. D. Blaker

Staff Fellow

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Molecular Pharmacodynamics

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

0.5

PROFESSIONAL:

0.4

OTHER:

0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

It has been found that N⁶-cyclohexyl[³H]adenosine binding sites are not associated with axons or terminals of serotonergic, noradrenergic, and cholinergic neurons in the hippocampus but may be associated with intrinsic neurons of the hippocampus which do not appear to be innervated by noradrenergic, cholinergic or serotonergic axons. It has been shown that THIP-induced analgesia in the mouse hot plate test does not involve an interaction with opiate receptors, bicuculline-sensitive GABA receptors, or the recognition site for baclofen. However, activation of serotonergic receptors potentiate the opiate- or THIP-induced elevation in nociceptive threshold. Finally, it has been demonstrated that ethanol specifically reduces the turnover rate of acetylcholine in the cortex and at the same time the body temperature is reduced. Maintenance of normal body temperature reverses the reduction in acetylcholine turnover.

Proposed Course:

This project was done in collaboration with D.L. Cheney, Chief of the Section on Molecular Pharmacodynamics and T.F. Murray, PRAT Fellow, SMRP, NIMH.

This project has been terminated.

Publication:

Murray, T.F., and Cheney, D.L.: Neuronal location of N⁶-cyclohexyl ³H-adenosine binding sites in rat and guinea pig brain. Neuropharmacology 21: 575-580, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01509-13 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Psychopharmacological studies of acetylcholine turnover: Behavior		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) W.D. Blaker Staff Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Pharmacodynamics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.0	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The behavioral and biochemical effects of GABAergic drugs on the septal-hippocampal system was studied by comparing the acetylcholine turnover rate (TR_{ACh}) in the rat hippocampus with extinction of a food reinforced lever press response after intraseptal injection of such drugs. Muscimol, a GABA_A agonist, produced a dose dependent and simultaneous increase in responding during extinction and decrease in TR_{ACh}. Bicuculline, a GABA_A antagonist, in equivalent doses, had the opposite behavioral effect, i.e. a general decrease in responding. Baclofen, a GABA_B agonist, had behavioral effects similar to those produced by muscimol, but was 5-10 times more potent and did not alter the hippocampal TR_{ACh}. Three to five weeks after bilateral injection of kainic acid into the hippocampus, there was a significant loss of bicuculline-insensitive GABA binding (GABA_B sites) in the septum, but no loss of baclofen insensitive GABA binding (GABA_A sites). This loss is presumably due to the degeneration of the glutamatergic hippocampal pyramidal cells which have terminals in the lateral septum. We hypothesize that both muscimol and baclofen exert their behavioral effects by decreasing the functional output of the hippocampus, muscimol by decreasing the stimulatory input to the hippocampus, and baclofen by presynaptically inhibiting the hippocampal efferents to the septum. </p>		

Project Description:

This project was done in collaboration with D.L. Cheney, Chief of the Section on Molecular Pharmacodynamics, SMRP, NIMH.

The present studies were undertaken to compare the biochemical and behavioral effects of GABAergic drugs on the rat septal hippocampal system. Recent evidence has pointed to the existence of two classes of GABA receptors in the CNS; a GABA_A receptor toward which muscimol is an agonist and bicuculline is an antagonist, and a GABA_B receptor toward which baclofen is an agonist. The GABA_A receptor is thought to be the "classical" GABA receptor which is coupled to Cl⁻ channels and whose activation decreases cell firing. The GABA_B receptor is much less characterized but has been implicated in the presynaptic inhibition of excitatory amino acid release. GABAergic drugs selective for either GABA_A or GABA_B sites were used in this study.

Numerous studies in the literature point to the involvement of the cholinergic septal hippocampal system in response inhibition, and thus extinction of a food reinforced level press response was used as a behavioral indicator of proper functioning of this system. The turnover rate of acetylcholine in the hippocampus was used as a biochemical indicator of the cholinergic activity of septal projections to the hippocampus. In such a procedure, phosphoryl(³H₉)choline was infused via the tail vein and the incorporation of label into choline and acetylcholine was determined using gas chromatography-mass fragmentography. From the choline and acetylcholine curves representing the change with time of the incorporation of the label, the fractional rate for acetylcholine efflux was determined. The fractional rate constant multiplied by the steady state concentration of acetylcholine yielded the turnover rate of acetylcholine.

The experimental protocol called for rats to be implanted with chronic septal cannulae and then trained on a continuous reinforcement scheduled over several days. The animals were then injected with various amounts of the appropriate drug via the septal cannulae and 20 minutes later challenged with extinction of the learned response for 10 minutes. This was immediately followed by the infusion of phosphoryl(³H₉)choline via the tail vein for the turnover rate determination.

It was found that acute injection of 0.3 to 3 nmoles of muscimol, a GABA_A agonist, decreased the turnover rate of acetylcholine in the hippocampus and increased responding during extinction when compared to saline-injected controls. These effects occurred simultaneously on a dose response curve. The GABA_A antagonist bicuculline decreased responding when administered at a dose of 1 nmole, having a behavioral effect opposite to that of muscimol. Baclofen, a GABA_B agonist, was found to increase extinction responding at doses of 0.05 to 0.2 nmoles. However, in contrast to muscimol, baclofen did not produce changes in the hippocampal acetylcholine turnover rate, even when given in near sedative doses.

Since it is known that the cholinergic connections from the medial septum to the hippocampus terminate on glutamatergic pyramidal cells which in turn project to the lateral septum, the neuronal localization of septal GABA_A and GABA_B binding sites was examined. Kainic acid was injected bilaterally into the hippocampus and 3-5 weeks allowed for complete degeneration of hippocampal efferents. [³H]GABA binding assays were then performed on thoroughly washed septal membranes under conditions to distinguish binding to GABA_A and GABA_B sites. Kainic acid treatment was found to decrease GABA_B type binding by ~40% while having no effect on GABA_A binding. This suggests that a sizable

proportion of GABA_B sites in the septum are located presynaptically on afferents from the hippocampus. It thus seems probable that muscimol and baclofen both exert their behavioral effects by inhibiting the output of the hippocampus, muscimol by inhibiting the stimulatory input to the hippocampus via the activation of GABA_A receptors and baclofen by presynaptically inhibiting hippocampal efferents via activation of GABA_B receptors.

These studies thus shed light on specific biochemical and behavioral consequences of administration of baclofen, a drug which although used clinically as an antispastic drug for some time, has been the subject of relatively few studies to elucidate biochemical sequelae of its behavioral effects in the CNS.

Future studies will involve the extension of the above behavioral and biochemical approaches to the study of another forebrain cholinergic system, the substantia innominata-cortical pathway whose degeneration has been implicated as a causative factor in Alzheimer's disease.

Publication:

Blaker, W.D., Cheney, D.L., Gandolfi, O., and Costa, E.: Simultaneous modulation of hippocampal cholinergic activity and extinction by intraseptal muscimol. J. Pharmacol. Exp. Ther. 225: 361-365, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZOI MH 01510-08 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Effect of cannabinoids on cholinergic and GABAergic dynamics in rat brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) A.V.Revuelta Visiting Associate SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Pharmacodynamics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.3	OTHER: 0.1
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Administration of the synthetic, crystalline <u>cannabinoid</u> , <u>nabilone</u> , and the potent dimethylheptyl derivative of (-)-delta-8-tetrahydrocannabinol reduces the turnover rate of <u>acetylcholine</u> in the <u>hippocampus</u> and reduces the turnover rate of <u>GABA</u> in the <u>septum</u> . As a working hypothesis we suggest that a group of inhibitory GABA-containing interneurons impinges on a second smaller group of inhibitory GABA containing interneurons which modulates the activity of cholinergic cell bodies located in the medial septum whose long axons project to the hippocampus.		

Proposed Course:

This project was done in collaboration with D.L. Cheney, Chief of the Section on Molecular Pharmacodynamics, SMRP, NIMH.

This project has been terminated.

Publication:

Revuelta, A.V., Cheney, D.L. and Costa, E.: The dimethylheptyl derivative of (-)-delta-8-tetrahydrocannabinol reduces the turnover rate of gamma-aminobutyric acid in the septum and nucleus accumbens. Life Sciences 30: 1841-1846, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01512-10 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Transmitter interactions in the regulation of pituitary function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> A. Guidotti Chief, Section of Neuroendocrinology SMRP NIMH		
COOPERATING UNITS (if any) L. Grandison, Dept. Physiology and Biophysics, College of Medicine and Chemistry, Rutgers Medical School, Piscataway, N.J.		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>The role of GABA, catecholamine and endorphins in the response of hypothalamus to different psychoactive drugs was studied by monitoring pituitary hormone release. Endogenous opiates stimulate PRL and block FSH release by activation of hypothalamic opiate receptors. These opiate receptors may be located on cells containing prolactin releasing factors since median eminence DA neurons or serotonergic neurons apparently do not mediate the effects of morphine. Stimulation of hypothalamic or brain GABA receptors with muscimol fails to block morphine or haloperidol-induced PRL release. In contrast stimulation of GABA receptors in anterior pituitary prevents the morphine or haloperidol-induced PRL release. GABA receptors with characteristics similar to those of rat were observed also in human anterior pituitary. This suggests a possible physiological role of pituitary GABA receptors in the release of PRL.</p>		

Proposed Course:

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01514-11 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Trans-synaptic control of protein synthesis		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) H. Kageyama Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In primary culture of adrenal chromaffin cells, addition of 8-Br-cAMP produces an induction of TH and a two- to three-fold increase in synthesis of enkephalin-like immunore-active material (ELM). TH and ELM induction is preceded by an activation of cytosol cAPK and by an increase in nuclear protein phosphorylation. The induction of TH, the increase of ELM and the increase of nuclear phosphorylation require that the assembly of microtubular proteins be functional. Anti-microtubular drugs such as colchicine and vinblastine (10^{-7} M) can block the TH and ELM induction elicited by 8-Br-cAMP, when the drugs are added less than 15 hours after 8-Br-cAMP. Since colchicine, added with cAMP also prevents the increase in nuclear phosphorylation, it is possible that the assembly of microtubular proteins might be operative in the intracellular translocation and nuclear uptake of catalytic subunits of cAPK activated by the addition of 8-Br-cAMP. In addition, these data support the view that an increase in nuclear protein phosphorylation is an essential step in the mediation of the acceleration of mRNA synthesis and the subsequent increase in TH and ELM synthesis elicited by 8-Br-cAMP. </p>		

Project Description:

This project was done in collaboration with A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Our objective was to study the molecular mechanisms whereby transsynaptic stimuli induce new synthesis of specific proteins in chromaffin cells of adrenal medulla. In previous studies we reported that in chromaffin cells of rat adrenal medulla, the sequence of molecular events whereby transsynaptic mechanisms regulate the tyrosine hydroxylase (TH) gene expression includes an increase in the cAMP/cGMP concentration ratio, an activation of cAMP-dependent protein kinase (PK) in cytosol and the translocation of the low molecular weight catalytic subunit of this protein kinase from the cytosol to the subcellular particles. The PK of nuclei is not regulated by cAMP but it increases during the transsynaptic induction of TH because the cAPK catalytic subunits translocate from cytosol to the nucleus. Thus, the activation and translocation of PK, triggered by the initial increase of cAMP, acts as a long range messenger for the transsynaptic expression of the genetic coding for TH.

Recently it has been reported that neuroactive substances coexist in the same axon terminals with the primary transmitter and upon release can function as cotransmitters modulating the gain of the primary transmitter at the receptor. A typical example of this coexistence is in the adrenal medulla cells where opiate peptides coexist with catecholamines and are released with these amines during transsynaptic regulation.

The question has been asked of whether in the adrenal cells the opiate peptides can undergo long term adaptive changes similar to the one reported for TH. Indirectly this question allows one to explore whether genes that control transmitter and cotransmitter synthesis are under a common regulatory process. Until recently interest in cotransmitter peptides has been focused primarily on their synthesis and processing and not on the regulation of gene expression. However, taking advantage of the development of recombinant DNA technology and in situ hybridization histochemistry, a better understanding of the regulation of the genes coding for these modulators will be forthcoming. In our study we have then decided to use as an in vitro system primary cultures of cow adrenal medulla cells. These cells were used as a model to evaluate the ability of 8-Br-cyclic AMP (8-Br-cAMP) to induce TH an enkephalin-like immunoreactive material (ELM) and to study the role of cAPK in this induction. This cell culture maintains a constant level of cyclic nucleotides, catecholamines, ELM and related enzyme activities for about four weeks.

Exposure of the cells to 8-Br-cAMP produces 48 hrs later, a dose related longlasting increase in TH and ELM activity; 8-Br-cGMP fails to modify TH and ELM. The increase in TH activity caused by 8-Br-cAMP is due to an increase of the V_{max} and in the number of enzyme molecules; the increase in ELM is due to an elevation of high and low MW opiate peptides and is preceded by an increase of proenkephalin mRNA as determined by Drs. Tang, Quach and Schwartz (see annual report) using complementary DNA. Both increases are preceded by an activation of cytosol cAPK associated with a decrease of the total cytosol cAPK. A sustained increase in nuclear phosphorylation begins 8 to 12 hrs after 8-Br-cAMP application. The delayed increase in TH and ELM activity induced by 8-Br-cAMP is blocked by actinomycin D, cycloheximide, colchicine and vinblastine.⁹ This reduction of TH and ELM induction elicited by colchicine and vinblastine (10^{-6} M) is observed only when these inhibitors of the microtubular protein polymerization were added 4 to 12 hrs after the addition of 8Br-cAMP which is the inducing stimulus. The addition of

colchicine 15 hrs after 8-Br-cAMP fails to inhibit TH or ELM activity. This blockade is associated with an inhibition of the increase in nuclear phosphorylation, but is not associated with an inhibition of protein synthesis. The increase of endogenous cAMP and the induction of TH were also produced by cholera toxin. These results suggest that the concomitant increase of TH and ELM elicited by 8-Br-cAMP is mediated by the translocation of cAPK subunits from cytosol to the nuclei and that this translocation requires the function of the microtubular network.

Since adrenergic mechanisms and opiate peptides have been implicated in the etiology of affective disorders an understanding of the molecular nature of the regulation of the biosynthesis of catecholamines and enkephalins may contribute to a better understanding of the synaptic defects that may be operative in the etiology of mental diseases. In addition, the translocation of cAPK subunits from the cytosol to the nuclei may operate as a basic mechanism in memory and/or learning.

In future studies, we plan to explore how the increase in nuclear phosphorylation regulates the expression of the gene coding for the induction of TH and ELM.

Publication:

Costa, E., Guidotti, A., Hanbauer, I., Kageyama, H., Kataoka, P., Panula, P., Quach, T.T., and Schwartz, J.P.: Adrenal medulla: Regulation of biosynthesis and secretion of catecholamines and enkephalins. In Usdin, E. (Ed.): Catecholamines. In press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01515-10 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical changes in cerebellum after treatment with psychoactive drugs		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
D.S. Shah	Visiting Associate	SMRP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Biochemically, the <u>cerebellum</u> is characterized by a large concentration of <u>cGMP</u>, <u>cAMP</u>, and <u>cGMP-dependent protein kinase</u>. Drug-induced changes in <u>GABA receptor</u> function can be easily monitored by measuring changes in these biochemical parameters. It is suggested that <u>diazepam</u> and <u>muscimol</u> (a modulator and a direct GABA receptor agonist, respectively), modulate cerebellar function by altering the <u>cGMP</u> system. <u>Diphenylhydantoin</u> has its own receptor on cerebellar structures and this receptor is modulated by benzodiazepines. </p>		

Proposed Course:

This project was done in collaboration with J.P. Chambon, Guest Worker and A. Guidotti, Chief of the Section on Neuroendocrinology, SMRP, NIMH.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01516-10 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical pharmacology of minor tranquilizers		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) A. Novelli Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 0.7	OTHER: 0.5
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Behavioral studies have shown similarity of action between benzodiazepines (BDZs) and GABA mimetic drugs, indicating that the pharmacological action of BDZs is mediated through GABAergic synapses. The molecular composition of the GABA receptors was studied biochemically. Two separate binding sites (one for ³H-GABA and one for ³H-diazepam) were isolated by differential solubilization from rat brain homogenates with Triton X-100. Photo labeled benzodiazepine receptors were further purified by preparative SDS gel electrophoresis and reverse phase HPLC. This may represent a useful and rapid technique to obtain BZD receptors in almost pure form. Purification of BZD receptors may help to understand the molecular structure and function of the GABA receptor system.</p>		

Project Description:

This project was done in collaboration with A. Guidotti, Chief of the Section on Neuroendocrinology; D. Konkel, Chemist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Recent evidence from this laboratory has suggested that benzodiazepines, may exert a beneficial effect in psychiatric disturbances by a primary action on GABAergic transmission. The present project intends to elucidate the molecular mechanisms of action of benzodiazepines (BDZs).

In order to elucidate this problem we have decided to initiate studies devoted to the solubilization and partial purification from rat brain cortex homogenates of [^3H] γ -aminobutyric acid (GABA) and [^3H] diazepam recognition sites.

Results

A high percentage of GABA binding sites (virtually free of benzodiazepine binding sites) was solubilized from homogenates of rat brain cortex incubated at 0°C with 1% Triton X-100 and a mixture of protease inhibitors. A large proportion of benzodiazepine binding sites was solubilized in the absence of apparent GABA binding capacity by incubating crude synaptic membrane preparations at 37°C with 0.05% Triton X-100. The characteristics of these two solubilized binding sites resemble those of the membrane-bound binding sites. However, unlike the membrane-bound sites, solubilized GABA and benzodiazepine recognition sites have lost the ability to cross-react. Hence, solubilized benzodiazepine binding sites are insensitive to GABA stimulation, while solubilized GABA binding sites are no longer protected by the benzodiazepines against heat inactivation. These results indicate that GABA and benzodiazepine recognition sites reside in two different molecules which, when bound to membranes, can interact reciprocally and modulate their binding affinity for specific ligands.

Isolation and purification of BZD recognition sites was attempted by photo labeling the BZD receptor with ^3H -flunitrazepam. Purification of the photolabeled receptor was achieved by preparative SDS gel electrophoresis followed by reverse phase HPLC. After HPLC chromatography the purity of the material was checked by two dimensional polyacrylamide gel electrophoresis. This technique allows a rapid purification of two major bands of proteins labeled by flunitrazepam.

After purification of BZD receptor to homogeneity we plan to analyze its amino acid composition and prepare monoclonal antibody. The antibody should help us to establish the interaction of this BZD recognition site with GABA recognition sites and whether benzodiazepine or beta-carboline binds to the same or two different recognition sites.

Benzodiazepine receptors are involved in regulation of anxiety, convulsions and sleep. A better understanding of these receptors may be of great value in determining the role of recognition sites of benzodiazepines in anxiety and in developing new drugs which may have selective action in this psychoaffective disorders.

Publication:

Guidotti, A.: Molecular mechanisms in the interaction between benzodiazepine and γ -aminobutyric acid receptors. In Yoshida, H., and Yamamura, H. (Eds.): Pharmacologic and Biochemical Aspects of Neurotransmitter Receptors. New York, Wiley & Sons Inc., 1983, pp. 267-274.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01518-07 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983.		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> The study of mammalian GABAergic and glutamatergic mechanisms		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> J. Wroblewski Visiting Fellow SMRP NIMH		
COOPERATING UNITS <i>(if any)</i> None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Pharmacodynamics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> ¹³ C-Proline and ² H-L-glutamic acid were injected intracerebroventricularly and the incorporation of the label from these compounds into proline and/or glutamate was examined. The maximal incorporation of label into striatal proline occurred 10 minutes after the injection. However, no label was observed in glutamate. The maximal incorporation of label from ² H-L-glutamate occurred within five minutes and declined thereafter. The amount of label was 3-fold higher in the <u>septum</u> than in <u>cortex</u> , <u>striatum</u> , and <u>hippocampus</u> .		

Proposed Course:

This work was done in collaboration with D.L. Cheney, Chief of the Section on Molecular Pharmacodynamics, SMRP, NIMH.

This project has been terminated.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01521-08 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Functional role of substance P and other peptides in nervous system		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J.P. Schwartz Research Chemist SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.2	OTHER: 0.2
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In studies on the function of <u>substance P</u> and on the effect of drugs on its distribution, the possibility that a pool of <u>substance P precursor</u> exists must be considered. The <u>chick embryo dorsal root ganglion</u> contains a molecular species of high molecular weight immunoreactivity which could function as a precursor in the formation of substance P. The content of this possible precursor is regulated by treatment of ganglia with <u>nerve growth factor</u>. Substance P, and its apparent precursor, have also been found in superior cervical ganglia, probably located in interneurons, as well as in many other tissues. Exposure of animals in utero to <u>anti-NGF antiserum</u> results in a loss of substance P from ganglia, spinal cord and skin, in agreement with a loss of DRG neurons. Adults exposed to anti-NGF show comparable losses of substance P content without a change in cell number. </p>		

Project Description:

Evidence is accumulating that many peptides, as well as protein hormones and transmitters, are synthesized as inactive precursors, and that the conversion of the precursor to the active component is an important step in the regulation of the modulatory peptide. We have used chick embryo dorsal root ganglia, which contain substance P cell bodies, to search for a precursor to substance P. In order to study the metabolism, as well as the development, of various peptidergic neurons, we have used animals exposed to antiserum against NGF. In animals exposed in utero or as newborns, there is a loss of substance P-containing cells from sensory ganglia, with a corresponding depletion of substance P in the spinal cord and skin. Preliminary results show the same sort of changes for somatostatin, another putative transmitter in sensory ganglia. In adult animals, in contrast, there is a depletion of the substance P content of these tissues with no loss of cell number. The effect of anti-NGF in the adult animals is surprising since sensory ganglia have been thought to lose their NGF responsiveness during embryological development. Studies with the anti-NGF treated animals have shown that substance P-containing neurons in adrenal medulla and ileum are also NGF-responsive, whereas those of the submaxillary gland, the retina, and a variety of brain regions are not. Anti-NGF and NGF have similar effects on the substance P content of cultured human fetal sensory ganglia. In addition to looking at other peptides, use of these animals allows us to examine interactions between comodulators and between neurons.

We plan to continue these studies by measuring other peptides in order to determine how wide-spread the dependence on NGF is. In addition, we will use the animals to examine interactions between comodulators and between neurons. For example, although loss of substance P-terminals in the spinal cord had no significant effect on opiate binding, recent immunohistochemical results suggest that GABA binding may change. The potential role of peptides as neurotransmitters and/or neuromodulators in the nervous system has expanded our knowledge of how the brain functions but has also expanded the possible sites where defects or altered metabolism could result in mental disorders. It thus becomes imperative to learn as much as possible about this new class of neuroactive compounds.

Publications:

Schwartz, J.P., Pearson, J., and Johnson, E.M.: Effect of exposure to anti-NGF on sensory neurons of adult rats and guinea pigs. Brain Res. 244: 378-381, 1982.

Baron-Van Evercooren, A., Kleinman, H.K., Ohno, S., Marangos, P., Schwartz, J.P., and Dubois-Dalq, M.E.: Nerve growth factor, laminin, and fibronectin promote neurite growth in human fetal sensory ganglia cultures. J. Neurosci. Res. 8: 179-193, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01524-08 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Evidence for peripheral dopaminergic neurons

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

M. Economou-Hadjiconstantinou

Guest Worker

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

0.6

PROFESSIONAL:

0.6

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

We previously provided experimental evidence that many peripheral tissues contain rather high concentrations of dopamine suggesting that it may be a neurotransmitter in addition to being a precursor for norepinephrine. Our current objective is to provide evidence that the cardiovascular system contains dopaminergic neurons.

Project Description:

This work was done in collaboration with N.H. Neff, Chief of the Section on Biochemical Pharmacology, SMRP, NIMH.

There is now substantial clinical and experimental evidence that dopamine receptors are found in the cardiovascular system. Activation of these receptors *in vivo* results in increased perfusion of some organs and a fall of blood pressure. Our objective is to provide evidence that the cardiovascular receptors are associated with dopaminergic neurons.

Blood vessels were dissected from rats and assayed for catecholamines by HPLC with electrochemical detection.

For most arterial vessels, dopamine content represented between 5-8 percent of the norepinephrine content. In contrast, for venous vessels, dopamine represented between 1-3 percent of norepinephrine. Following treatment with the neurotoxin 6-hydroxydopamine, both norepinephrine and dopamine content declined. If norepinephrine neurons were protected by administering the uptake blocking drug, desipramine, before 6-hydroxydopamine treatment, only dopamine content fell. Moreover, with the specific noradrenergic neurotoxin DSP-4 (N-(2-chloroethyl)N-ethyl-2-bromobenzylamine) norepinephrine content fell but not dopamine content. Thus, norepinephrine and dopamine appear to be contained in separate structures which can be pharmacologically manipulated independently. These observations support the hypothesis that a peripheral dopaminergic neuronal system innervates peripheral dopaminergic receptors.

Schizophrenia and Parkinsons disease are associated with abnormal metabolism of dopamine. Some of the symptoms of these diseases may be related to a deficiency of peripheral dopaminergic neurons. Moreover, some of the side effect of drugs that act on the central dopaminergic system may be the consequences of actions on peripheral dopaminergic neurons or their receptors. Our studies are an attempt to answer some of these important questions.

Future studies will be directed towards the histological identification of peripheral dopaminergic neurons.

Publications:

Relja, M., Lackovic, Z., and Neff, N.H.: Evidence for the presence of dopaminergic receptors in vas deferens. Life Sci. 32: 1565-1674, 1982.

Neff, N.H., Karoum, F., and Hadjiconstantinou, M.: Dopamine-containing small intensely fluorescent (SIF) cells and sympathetic ganglion function. Fed. Proc., in press.

Lackovic, Z., and Neff, N.H.: Minireview: Evidence that dopamine is a neurotransmitter in peripheral tissue. Life Sci. 32: 1665-1674, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01525-07 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of gene expression and protein synthesis of neural tissues		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) I. Mocchetti, Guest Worker; T. Quach, Visiting Associate SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.5	PROFESSIONAL: 1.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unredacted type. Do not exceed the space provided.) <p>Rat <u>C6 glioma</u> cells contain a beta-adrenergic receptor, stimulation of which leads to an induction of a specific form of cyclic nucleotide phosphodiesterase (PDE) and an increase of nerve growth factor content. The induction of PDE is shown to require a rise in cyclic AMP, activation of cAMP-dependent protein kinase, translocation of the catalytic subunit of the kinase to the nucleus, phosphorylation of non-histone chromosomal proteins, and RNA polymerase II. Inhibition of one of these steps by drugs such as colchicine, cordycepin or alpha-amanitin prevents PDE induction. Direct measurement of mRNA levels can be made using cDNA probes. Use of a cDNA probe coding for human pheochromocytoma <u>proenkephalin</u> (PE) has shown that rat brain makes a very similar protein. Chronic treatment of rats with haloperidol for 2-3 weeks leads to a specific increase in striatal PE mRNA with no change occurring in other brain regions. Treatment of bovine <u>adrenal chromaffin</u> cells with 8-Br-cyclic AMP results in an increase of PE mRNA in these cells, which is time- and dose-dependent and not replicated by 8-Br-cyclic GMP. There is a comparable change in the content of enkephalin-like peptides.</p>		

Project Description:

This project was done in collaboration with J.P. Schwartz, Research Chemist; E. Costa, Chief of the Laboratory of Preclinical Pharmacology; P. Onali, Visiting Fellow and F. Tang, Guest Worker, SMRP, NIMH.

The nervous system-derived cell line C6 glioma contains a beta-adrenergic receptor through which cyclic AMP-dependent functions in the cell can be regulated. Among the consequences of isoproterenol activation of adenylate cyclase in the C6 glioma cells are an induction of cyclic AMP phosphodiesterase (PDE) and an increase in nerve growth factor content. The increase of PDE is a process which reaches a peak in 3-4 hrs and requires new protein synthesis. The cell cytoplasm contains 2 forms of PDE, which are separable on a DEAE-Sephacel column. The first form utilizes both cyclic AMP and cyclic GMP as substrates and is activated by calcium and calmodulin. The second form, which acts only on cyclic AMP, is specifically induced by isoproterenol treatment. This form is not affected by either calcium plus calmodulin or cyclic GMP (up to 100 μ M). Further purification revealed a single peak of activity with an apparent MW of 54,000, whose specific activity is increased following beta-adrenergic stimulation. Kinetic analysis revealed a non-linear Hofstee plot with apparent K_m values of 2-5 μ M for cyclic AMP.

Characterization of protein kinase activation is the first step in determining how the cyclic AMP-activated protein kinase is increasing the beta-NGF and PDE content of the cells. Following activation of the cyclic AMP-dependent protein kinase, there is a translocation of the catalytic subunit of the kinase from the cytosol to the nucleus. Pretreatment of glioma cells with vinblastine or colchicine blocks the increase of nuclear protein kinase and the increase of PDE activity elicited by isoproterenol. These results suggest first that the translocation of activated subunits of protein kinase from cytosol to the nucleus is required for the induction of new synthesis of PDE molecules. In addition, since vinblastine and colchicine inhibit microtubule polymerization, the results suggest that microtubules are involved in the translocation process. Regulation of protein phosphorylation depends on activation of protein kinase, location of the activated enzyme, and specific substrates present in the sites where activated enzyme is located. An as yet unidentified nuclear acidic protein(s) represents a substrate for the translocated kinase. At 1-2 hrs following isoproterenol, there is increased phosphorylation of the acidic protein fraction, with no change in the degree of phosphorylation of either histones or the remainder of the nuclear proteins. Both the increased phosphorylation of acidic proteins and the PDE induction can be blocked by cordycepin, suggesting that acidic proteins regulate expression of the gene for PDE. RNA polymerase II is also required for induction of PDE. Its activity in vivo and in vitro as well as the induction of PDE can be blocked by either actinomycin D or alpha-amanitin.

Since neither cycloheximide nor vinblastine affect the increase in beta-NGF content elicited by isoproterenol, it is inferred that at least the early (3 to 6 hr after isoproterenol) increase in NGF is not the result of new protein synthesis. We hope to obtain a cDNA for the gene for NGF and propose to use it to determine whether cyclic AMP regulates long-term expression of the mRNA for NGF in C6 cells, and how the precursor pro-NGF is biosynthetically regulated.

In parallel with these studies, we have undertaken a series of experiments using a cDNA coding for human pheochromocytoma proenkephalin as a probe in order to determine whether different tissues contain multiple genes and/or multiple mRNAs encoding proenkephalin.

In the first series of experiments, we have demonstrated the presence of proenkephalin mRNA in seven regions of rat brain, thus showing that the proenkephalin present in brain is essentially identical to that in adrenal medulla. Chronic treatment of rats with the antipsychotic haloperidol for 2-3 weeks causes a specific four-fold increase in proenkephalin mRNA in striatum with no change in other regions, which correlates with a two-fold change in the content of enkephalin-like peptides. Similar changes are seen one week following reserpine administration. We plan to continue these studies by looking at other drugs such as sulpiride and 6-hydroxy-dopamine. These studies demonstrate that certain neuroactive drugs can affect gene expression.

We have also initiated a series of experiments utilizing bovine adrenal chromaffin cells. Treatment of the cells with 8-Br-cyclic AMP results in increased expression of proenkephalin mRNA within one day. The effect is dose-dependent but not reproduced by 8-Br-cyclic GMP. Changes in the mRNA content are followed in time by changes in the cellular content of enkephalin-like peptides as well as high MW forms of enkephalin, and increased release to the medium of the peptides.

Previous work in the laboratory has shown a role for cyclic AMP-dependent protein kinase translocation to the nucleus in the induction of tyrosine hydroxylase in the cells. We now plan to look at the role of the protein kinase and of nuclear protein phosphorylation in the regulation of proenkephalin mRNA content of the chromaffin cells. In addition we will study the effects of other opiates and neurotransmitters. This model system allows us to ask questions about the regulation of expression of the cotransmitters, catecholamines and enkephalin peptides.

Both neurotransmitters and drugs have certain long-term effects or effects which appear only after chronic exposure. Some of these effects occur at the level of transcription and the technique of molecular biology utilizing specific gene probes will enable a better understanding of changes occurring in the brain as a result of either chronic drug treatments or of disease-induced changes in transmitter release or function. Such an understanding will enable us to design better drugs or treatments for mental diseases.

Publication:

Tang, F., Costa, E., and Schwartz, J.P.: Increase of proenkephalin mRNA and enkephalin content of rat striatum after daily injection of haloperidol for 2 to 3 weeks. Proc. Natl. Acad. Sci., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01526-07 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Retina: A model for studying synaptic biochemistry		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. Economou-Hadjiconstantinou Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) Dr. J. Cohen, Dept. Pharmacology, Howard Medical School, Washington, D.C. Dr. A. Mariani, NEI, Bethesda, M.D.		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Biochemical Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Our present interest is to investigate some of the properties of the epinephrine-containing system of <u>retina</u> and how epinephrine-containing neurons interact with <u>dopamine-containing</u> neurons.		

Project Description:

This project was done in collaboration with N.H. Neff, Chief of the Section on Biochemical Pharmacology and P. Panula, Visiting Fellow, SMRP, NIMH.

Dopamine, norepinephrine and epinephrine are present in mammalian retina (Z01 MH 01526-06 SMRP). Dopamine is the major catecholamine while norepinephrine and epinephrine represent about 5 percent of the dopamine content. Dopamine is contained in a subpopulation of amacrine cells and has been the subject of numerous studies by our laboratory in the past. We have now investigated the origin and properties of the norepinephrine and epinephrine of retina.

Catecholamines were assayed by HPLC with electrochemical detection. Phenylethanolamine-N-methyltransferase (PNMT) activity was evaluated by methylating various substrates using S-adenosylmethionine-³H as the methyl donor.

Following superior cervical ganglionectomy the content of norepinephrine declined in the retina but epinephrine and PNMT activity did not. This suggests that norepinephrine is associated with the sympathetic neurons that innervate retinal tissue perhaps being present on the blood vessels. In contrast, epinephrine and PNMT are intrinsic to the retina. PNMT of retina has many of the substrate specificity and inhibitor sensitivity characteristics of PNMT of brain. Moreover, enzymatic activity was enhanced in newborn rat retina by treatment with dexamethasone, a finding that is similar to the enzyme activity of brain. Exposure of animals to a lighted environment increased retinal epinephrine suggesting that it plays a role in vision.

In subsequent studies, PNMT-like immunoreactivity was localized to a subclass of amacrine cells whose catecholamine content could only be observed with histofluorescent procedures if animals were treated with a monoamine oxidase inhibitor. Thus, there appears to be at least two classes of catecholamine-containing amacrine cells: dopamine- and epinephrine-containing amacrine cells. Moreover, when viewed in the light microscope, the processes of the epinephrine amacrine cells appeared to be closely associated with the dopamine amacrine cells.

In another series of studies, the possible interaction of the two catecholamine amacrine systems was evaluated. In the dark, when dopamine metabolism by the retina is at a low level, alpha-2 adrenoceptor antagonists activated the metabolism of dopamine in retina. Alpha-2 agonist drugs were inactive if the animals are maintained in the dark. In the light when dopamine metabolism in the retina is accelerated alpha-2 agonists are able to reduce dopamine metabolism to values found normally in the dark. Apparently, epinephrine amacrine cells of retina play a role for inhibiting dopamine metabolism in the dark and activation in the light may be, in part, the consequence of disinhibition of dopamine amacrine cells by epinephrine amacrine cells. Our studies taken together suggest that retina has epinephrine-containing amacrine cells and these cells, via alpha-2 receptors, modulate the metabolism of dopamine amacrine cells.

Many of the drugs that are used to treat human mental disorders modify catecholaminergic neuronal function. Our studies are providing the bases for understanding the pharmacology of these drugs as well as the side effects associated with therapy.

Our future efforts will be directed towards understanding the molecular events that occur at neuronal membranes for the modulation of dopaminergic neurons.

Publications:

Cohen, J., and Neff, N.H.: Activation of retinal tyrosine hydroxylase: Tolerance induced by chronic treatment with haloperidol does not modify the response to light. J. Pharmacol. Exp. Ther. 221: 326-328, 1982.

Iuvone, P.M., Rauch, A.L., Marshburn, P.B., Glass, D.B., and Neff, N.H.: Activation of retinal tyrosine hydroxylase in vitro by cyclic AMP-dependent protein kinase: Characterization and comparison to activation in vivo by photic stimulation. J. Neurochem. 39: 1632-1640, 1982.

Cohen, J., Hadjiconstantinou, M., and Neff, N.H.: Activation of dopamine-containing amacrine cells of retina: Light-induced increase of acidic dopamine metabolites. Brain Res. 260: 125-127, 1983.

Hadjiconstantinou, M., Cohen, J., and Neff, N.H.: Epinephrine: A potential neurotransmitter in retina. J. Neurochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

PROJECT NUMBER

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZOI MH 01527-07 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Studies of endogenous opioids using HPLC

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

J.L. Meek

Pharmacologist

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Group on High Pressure Liquid Chromatography

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

0.1

PROFESSIONAL:

0.1

OTHER:

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unrounded type. Do not exceed the space provided.)

The object of this study was to develop derivatizing reagents for peptides which could increase the sensitivity of detection of peptides by HPLC. This work, which was completed during the previous fiscal year, lead to discovery of 2 reagents which can improve sensitivity by 50-500 fold for simple peptides. Utilization of these reagents will await future work to improve separation conditions and sample cleanup. After preparation of a description of the work for publication, this project was terminated.

Proposed Course:

This project has been terminated.

Publication:

Meek, J.L.: Derivatizing reagents for HPLC detection of peptides at the picomole level. J. Chromatogr., in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01531-06 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Nerve growth factors: synthesis and function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J.P. Schwartz Research Chemist SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.2	PROFESSIONAL: 1.2	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Recent evidence suggests that a family of nerve growth factors exist, each effective for a certain population of neurons. Human <u>fibroblasts</u> make a NGF similar to mouse submaxillary NGF in both its immunoreactive and biological properties. However, fibroblasts from patients with the genetically inherited disease <u>familial dysautonomia</u> contain a NGF which is immunoactive but has very low biological activity. Mouse brain contains a factor which is NGF-like by immunoassay but has no biological activity. This factor increases in the cerebella of the <u>pcd mutant mouse</u> as the Purkinje cells die out and astrocytes proliferate. The <u>PC12 pheochromocytoma cell</u> line has been used to study the biological effects of NGF and whether these effects require NGF-receptor endocytosis. <u>Transglutaminase</u> is present in the cells and can be induced by butyrate treatment - the effect of this treatment on the NGF response is being studied.</p>		

Project Description:

This project was done in collaboration with J. Byrd, Guest Worker, SMRP, NIMH.

Nerve growth factor (NGF), as isolated classically from the mouse submaxillary gland by Levi-Montalcini, is a protein required by certain populations of peripheral neurons for both survival and maintenance of function. Recent evidence suggests that many "nerve growth factors" exist, specific for different populations of neurons in either the CNS or PNS. A defect or loss of one of these factors would result in a disease of the nervous system.

We have carried out studies related to the NGF made by human fibroblasts taken from normal controls and from patients with the autosomal recessive disease familial dysautonomia. Our biochemical results suggest that the patients' cells make a defective NGF, detectable immunologically but with much less biological activity in the chick embryo dorsal root ganglion bioassay. Chemical characterization of the NGF present in the fibroblasts has proven to be extremely difficult because the NGF represents only 0.001% of the total cell protein. The recent announcement of the cloning of the gene for NGF has made available a cDNA probe which will allow direct examination of the structure of the human gene in both dysautonomic and control fibroblasts.

We have used an inbred mouse strain with a genetically inheritable neurological disease, the *pcd* mutant, in which Purkinje cells develop normally but die out from day 20-50 after birth, to ask whether the proliferating astrocytes produce a CNS "NGF". Our earlier work using a CNS-derived clonal glial cell line showed that these cells made NGF and that the amount could be regulated by beta-adrenergic agonists. Our results with the mice demonstrate that there is a protein present in cerebellum which shows immunological cross-reactivity with NGF but which has no biological activity in the classic bioassay. The amount of this "NGF"-like protein increases in *pcd* cerebellum as astrocytes are proliferating. We will use the cDNA probe for NGF under relaxed conditions to attempt to identify this CNS "NGF"-like protein in order to understand its role in the normal development of the CNS and specifically the cerebellum.

In order to understand the biological effects of NGF better, studies have been initiated using the PC12 pheochromocytoma cell line which has NGF receptors and responds to NGF biochemically and biologically. These studies are centered on two major questions: 1) is the internalization of NGF along with its receptor required for its biochemical effects; 2) does NGF stimulate a tyrosine protein kinase associated with its receptor in the cell membrane, as has been demonstrated for other peptide hormones such as EGF and insulin.

Because the enzyme transglutaminase (TGase) appears to be involved in many systems of receptor-mediated hormone endocytosis, we have measured this enzyme in PC12 cells and identified a series of inhibitors as well as an apparent inducer, butyrate, of the enzyme. We can thus manipulate the TGase activity up or down and ask whether this affects NGF endocytosis and ultimately responses of the cells to NGF. Understanding how NGF exerts its physiological effects will provide clues as to how both it and other "nerve growth factors" function and ultimately will lead to an understanding of the role these "NGFs" may play in human mental disorders.

Publications:

Schwartz, J.P., Ghetti, B., Truex, L., and Schmidt, M.J.: Increase of a nerve growth factor -- like protein in the cerebellum of PCD mutant mice. J. Neurosci. Res. 8: 205-211, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01532-06 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of catecholamine receptor		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) D.M. Chuang Chemist SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.4	PROFESSIONAL: 1.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The system of isolated frog erythrocytes has been used as a model to study the molecular mechanisms of the loss of membrane-bound β-adrenergic receptor recognition sites that occurs during receptor desensitization induced by exposure to excessive amount of isoproterenol. We have shown that the decrease in the membrane-bound β-receptor recognition sites is associated with an internalization of the surface bound receptors into subcellular structures. A portion of the internalized receptor site is present in a soluble fraction which is devoid of adenylate cyclase activity and guanine nucleotide binding protein. This soluble or internalized receptors bind β-receptor agonists with low affinity and this agonist binding is unaffected by guanine nucleotides. β-Receptor internalization is energy required and may involve the activity of transglutaminase and lysosomal enzymes. When β-receptors are resensitized the internalized receptors appear to be cycled to the plasma membrane. In an attempt to obtain an insight of the detailed mechanisms of β-receptor internalization, we have produce a monoclonal antibody to β_2-receptors of frog erythrocytes by using a clone of hybridoma derived from spleen cells of immunized mouse and myeloma P3 x 63.Ag8. This monoclonal antibody directs against a site that is distinct from the ligand binding site. It also immunoprecipitates internalized β-receptor recognition site in frog erythrocytes but this interaction is of relatively low affinity, suggesting that internalized and membrane-bound β-receptor sites differ in their biochemical properties. We are attempting to produce a library of β-receptor monoclonal antibodies in order to elucidate a structural and functional relationship of β-adrenergic receptors and to obtain a better understanding of the sequences of events that occur during β-receptor internalization. </p>		

Project Description:

This project was done in collaboration with Ora Dillon-Carter, Chemist, SMRP, NIMH.

It is well established that receptors for a variety of neurotransmitters become desensitized when they are over-stimulated by a receptor agonist. This receptor adaptation is considered to be vital for the regulation of receptor function and the synaptic plasticity in the mental health. Therefore a better understanding of the molecular mechanisms involved in the receptor desensitization could lead to a new therapy for some mental illnesses resulting from malfunction of a receptor. The system of isolated frog erythrocytes has been used as a model to study the molecular events that occur during desensitization of β -adrenergic receptor (BAR) induced by prolonged exposure (about 2 hrs) of erythrocytes to isoproterenol. This BAR desensitization is associated with a reduction in the number of membrane-bound BAR recognition sites as well as an attenuation of adenylate cyclase activity stimulated by BAR agonists. Thus the density of surface bound BAR appears to control the magnitude of the receptor response. We have provided evidence that the loss of membrane-bound BAR recognition sites is due at least in part to an internalization of BAR sites. This receptor internalization is reflected by an increase in the number of soluble BAR recognition site which is devoid of adenylate cyclase activity and guanine nucleotide binding protein. This soluble or internalized receptor site binds BAR agonists with low affinity and this binding is unaffected by guanine nucleotides as opposed to the membrane-bound BAR. BAR internalization is energy required and may involve the activities of transglutaminase and lysosomal enzymes. When BAR is resensitized following removal of isoproterenol from erythrocytes, the internalized BAR recognition site appears to be cycled to the plasma membrane to restore the functional sensitivity that is not during desensitization.

Although BAR internalization in frog erythrocytes has been confirmed by Lefkowitz and coworkers recently (J. Biol. Chem. 258:3032-3038, 6410-6414, 1983), the detailed mechanisms and the pathway of this event remain largely unclear. In an attempt to gain insight of the molecular mechanisms of BAR internalization, we have initiated an attempt to produce monoclonal antibodies against BAR recognition site prepared from frog erythrocytes (which belong to β_2 subtype). Mice (balb/c strain) were immunized with a partially purified preparation of BAR solubilized from frog erythrocyte membranes and the spleen cells from these immunized mice were further immunized in vitro with the immunogen in the presence of "conditioned thymus lymphocyte medium." The resulting spleen cells were then fused with a line of myeloma P3 x 63.Ag8 which secretes IgG₁K chains. Initial screening using ELISA (enzyme-linked immunosorbent assay) indicates that about 15-20% of the hybridomas formed produces antibodies against the immunogen. Specific screening for antibodies against BAR utilizes immunoprecipitation of BAR labeled with ¹²⁵I-iodohydroxybenzylpindolol, a high affinity specific ligand. Cells from positive wells are cloned and subcloned by limiting dilution to obtain a monoclonal culture and massive production of monoclonal antibodies is made by growing hybridomas as ascitic tumors in mice. A monoclonal antibody thus obtained was found to immunoprecipitate both BAR solubilized from plasma membranes of frog erythrocytes and those internalized into erythrocytes during isoproterenol-induced receptor desensitization. However the shape of the immunoprecipitation curve suggests that this monoclonal antibody has lower affinity for the internalized BAR. Such a difference is in line with our previous observations that internalized and plasma-membrane bound BAR differ in several respects in their biochemical properties (Mol. Pharmacol. 18:348-355, 1980). Binding inhibition studies indicate that this monoclonal antibody directs against a site distinct from the ligand binding site. Currently we are producing more monoclonal antibodies produced by hybridomas derived from myeloma P3 x 63.Ag8-653, a line that does not synthesize immunoglobulin, in

order to obtain a library of monoclonal antibodies to BAR. These antibodies will be used to localize BAR at the electron microscopic level in an attempt to understand the detailed pathway of BAR internalization. In addition these BAR monoclonal antibodies will be used to purify messenger RNA for BAR which will then be translated in vitro into BAR protein. Our future goal is to use these purified messenger RNA to clone the gene for BAR.

The finding that BAR can be internalized has provided a new basis for molecular mechanisms of receptor desensitization. Since certain mental illnesses are related to malfunction of neurotransmitter receptors, the study of the mechanisms of BAR internalization has added to the further understanding of the pathological basis of some mental diseases. It is anticipated that this study may eventually lead to a new therapy for these diseases. The methodology successfully employed in the production of BAR monoclonal antibodies may also be used to prepare monoclonal antibodies for other neurotransmitter receptors or neuropeptides and contributes to the understanding of the physiological role of these molecules.

Publications:

Chuang, D.M.: β -Adrenergic receptor internalization and processing: Role of transglutaminase and lysosomes. Molecular and Cellular Biochem., in press.

Chuang, D.M., Barbaccia, M.L., Brunello, N., and Kinnier, B.: Receptor regulation: An overview. In Hanin I. (Ed.): Dynamics of Neurotransmission. New York, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01536-05 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Characterization of receptors for putative neurotransmitters		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) D. Cavalla, Visiting Fellow; W. Wojcik, Staff Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Biochemical Pharmacology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The purpose of this project is to identify and study the <u>receptors of putative transmitter substances</u>. Our present objective is to: 1) develop <u>photoaffinity receptor binding ligands for adenosine receptors</u> and 2) determine if persistent activation of <u>muscarinic receptors</u> results in desensitization of the muscarinic inhibitory coupled <u>adenylate cyclase system of striatum</u>. </p>		

Project Description:

This project was done in collaboration with M.C. Olinas, Visiting Associate; P. Onali, Visiting Associate; N.H. Neff, Chief of the Section on Biochemical Pharmacology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Abnormal receptor function may play a role in the etiology of some forms of mental and neurological disorders. An understanding of receptor function may give insight into such disorders. Our immediate objective is: I) to develop photoaffinity receptor ligands for adenosine receptors and; II) to determine if persistent activation of muscarinic receptors in striatum results in desensitization of the muscarinic inhibitory coupled adenylate cyclase system.

- I. Azidoadenosine analogs were synthesized and evaluated for their ability to inhibit the specific binding of radioactive cyclohexyladenosine, an adenosine agonist analogue, to plasma membranes prepared from brain. The most active compound was 2-azidoadenosine which appeared to irreversibly bind to adenosine A₁ and adenosine A₂ receptors when incubated with membranes prepared from brain and exposed to ultraviolet radiation.

Some investigators have speculated that anxiolytic drugs act at adenosine receptor sites. Presently, the role of the adenosinergic system in neuronal function is unknown. Our goal is to provide information on the possible role of this system in health and disease. Future studies will deal with the synthesis of high specific radioactivity photoaffinity ligands which will be used to isolate and characterize adenosine receptors.

- II. Chronic inhibition of rat striatal acetylcholinesterase by treatment with diisopropyl-fluorophosphate (DFP) resulted in desensitization of muscarinic inhibition of striatal adenylate cyclase. The ability of acetylcholine to inhibit basal adenylate cyclase activity was reduced by about 50 percent. Moreover, the ability of acetylcholine to inhibit dopamine-activated adenylate cyclase was completely lost by the treatment. These studies are providing fundamental information on the mechanisms that are responsible for controlling receptor sensitivity to ligands and on the interaction of several receptors on the same membrane. These studies are of special importance because they deal with long term changes of receptor responsiveness and drug treatment.

Drugs that interact with dopaminergic receptors are used to treat a variety of mental health problems. Our goal is to determine the mechanism of action of these drugs and how new drugs might be modified to improve treatment. Future studies will be undertaken to determine if other receptors in brain also interact to control adenylate cyclase responsiveness.

Publications:

Olinas, M.C., Onali, P., Neff, N.H., and Costa, E.: Adenylate cyclase activity of synaptic membranes from rat striatum: Inhibition by muscarinic receptor agonists. Mol. Pharmacol. 23: 393-398, 1983.

Olinas, M.C., Onali, P., Neff, N.H., and Costa, E.: Muscarinic receptors modulate dopamine-activated adenylate cyclase of rat striatum. J. Neurochem., in press.

Hadjiconstantinou, M., and Neff, N.H.: Ascorbic acid could be hazardous to your experiments: A commentary on dopamine receptor binding studies with speculation on a role for ascorbic acid in neuronal function. Neuropharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01537-05 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT <i>(80 characters or less. Title must fit on one line between the borders.)</i> Biochemical pharmacology of GABA receptor system		
PRINCIPAL INVESTIGATOR <i>(List other professional personnel on subsequent pages.)</i> <i>(Name, title, laboratory, and institute affiliation)</i> B. Wise PRAT Fellow SMRP NIMH		
COOPERATING UNITS <i>(if any)</i> None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK <i>(Use standard unreduced type. Do not exceed the space provided.)</i> <p> <u>GABA-modulin</u>, an endogenous membrane protein, which inhibits non competitively ^3H-GABA binding to synaptic plasma membranes, has been isolated and purified to homogeneity using acidic extraction followed by Sephadex column purification and HPLC. <u>GABA-modulin</u> is a synaptosomal peptide of 16,000 MW which inhibits the binding of ^3H-GABA and the GABA-induced stimulation of ^3H-diazepam binding to synaptic membranes. The GABA-modulin molecule contains an abundance of hydrophilic residues (especially basic residues), and no cystein or GABA. End group analyses of GABA-modulin indicated that the carboxy terminus is free, while the N-terminus is blocked. GABA-modulin can be phosphorylated by cAMP and Ca^{++}-calmodulin dependent protein kinase. <u>Phosphorylation of GABA-modulin</u> by cAMP resulted in the lost of inhibition on GABA receptors. The role of <u>GABA-modulin</u> in the control of GABA receptor system function is presently being investigated. </p>		

Project Description:

This project was done in collaboration with D. Konkel, Chemist; A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

When GABA receptor sites are occupied by the endogenous agonist, the ion channel located in the membrane becomes permeable to Cl^- ion, resulting in a hyperpolarization or depolarization of the receptive neuron depending on the concentration of Cl^- in the surrounding medium. The goal of this project is to provide a better understanding of the function of the GABA receptor complex and in particular the coupling of GABA recognition site with the Cl^- channel as a first step in developing new potent and specific drugs which like benzodiazepines can facilitate the stimulation of GABA receptors.

Our studies indicate that freshly prepared crude synaptic membranes, purified synaptic plasma membranes, or membranes from neuroblastoma clonal cell lines, contain a protein inhibitor of ^3H -GABA binding which was termed GABA-modulin (GM). Repeated washing of these membranes combined with freezing, thawing and treatment with Triton X-100 removes GM and unmasks an additional population of GABA recognition sites characterized by high affinity for the agonist. Subjecting the Scatchard plot of these binding studies to the graphic analysis of Rosenthal for one ligand and two types of binding sites, the total density of GABA receptor sites and the relative portion of high (K_d 20-40 nM) and low affinity component (K_d 200-400 nM) of ^3H -GABA binding can be estimated.

GM was purified and characterized. The first step of the extraction procedure employed was homogenization of the tissue in hot (80°) 1 N acetic acid. This method was preferred to extraction procedures at a neutral pH because acid extraction at 80° destroyed proteolytic activity. The extraction in hot acetic acid was followed by 60% ammonium sulfate precipitation, Sephadex G-100 and G-75 column chromatography and anion exchange chromatography. Final purification was achieved by applying the material to a reverse phase HPLC Bio-Sil ODS-10 column.

Using this technique, the material was purified to homogeneity in a single (30 min chromatographic run. The major peak of protein eluting from the reverse phase HPLC with 50% acetonitrile inhibited the high affinity (K_D 20 nM) ^3H -GABA binding (IC_{50} 0.5 μM). This activity, which was destroyed by hydrolysis with trypsin or chymotrypsin, was not due to contaminating GABA, as amino acid analysis of the material did not detect any GABA. HPLC, with different columns and buffer conditions, polyacrylamide gel electrophoresis at different pH, analysis of amino acid composition, and carboxyl terminal amino acid analysis concurred to support the view that GM was purified to homogeneity. The molecular weight of GM evaluated by 12% SDS gel (17,000) is in good agreement with the molecular weight calculated from the results of the amino acid composition (16,200).

Amino acid composition, anion exchange chromatography and acrylamide gel with urea at acidic pH revealed that the protein is basic in nature. In washed Triton X-100 or AgNO_3 -treated crude synaptic membranes, purified GM inhibited both binding of ^3H -GABA to the high affinity site and GABA induced stimulation of ^3H -diazepam binding with an IC_{50} of around 0.5 μM . This concentration was within the range of GM concentration present in brain. In fact, it can be calculated from the recovery studies with ^{125}I -GM that the rat brain contained approximately 6 μM of GM. The action of GM is specific for GABA binding, because at doses up to 5 times higher GM failed to influence other ^3H -ligand binding. In addition, the specificity of GM action is confirmed by the lack of effect by other proteins

(histone small rat basic protein, lysozyme) of similar molecular weight and/or charge on ^3H -GABA binding. Studies with subcellular fractionations from brain homogenates or neuroblastoma membrane preparations indicates that GM is highly concentrated in synaptosomal membranes in close association with the recognition sites for GABA.

Purification of GM has opened new interesting research approaches for the studies of the regulation of GABAergic transmission. For example, we are presently studying if GM is the only brain peptide that inhibits ^3H -GABA binding. Although the activity of GM was destroyed by trypsin or chymotrypsin digestion, we could not exclude the possibility that the GM we had purified was the precursor of the endogenous modulator; in fact, we have not yet studied the activity of GM fragments produced by more limited proteolysis.

Another important question is whether GM facilitates or inhibits the biological activity of GABA released by nerve stimulation. Preliminary experiments show that when GM, which down regulates ^3H -GABA binding, is injected into the cerebral ventricles, it exacerbates the convulsions induced by isoniazid, suggesting that the increase in free GM decreases the action of GABA.

Finally, the question of the mechanism by which GM inhibits the binding of ^3H -GABA remains to be explored in detail. Because GM inhibits the high affinity GABA binding in an apparently noncompetitive fashion, it is proposed that the mechanism is primarily allosteric in nature. In this regard, the observation that GABA-modulin is a good substrate for phosphorylation is interesting. In fact Ca^{2+} and cAMP-dependent phosphorylation is operative in regulating the degree by which GM control GABA recognition site. When GM is extensively phosphorylated loses its ability to inhibit GABA binding.

Evidence has been accumulated to suggest that abnormalities in GABAergic function may be operative in determining the symptoms of Huntington's disease, Parkinson, epilepsy and possibly schizophrenia. Thus the biochemical and pharmacological characterization of GABA receptors modulation may be a relevant approach to find compounds which are capable of modifying the GABAergic system in man.

We are now developing monoclonal antibody against GM to measure GABA-modulin content in different biological samples and to understand whether drugs that interfere with the action of GABA-modulin may be useful in the control of the GABA receptor system in neurological and/or psychiatric disorders.

Publications:

Costa, E., Corda, M.C., Wise, B., Konkell, D., and Guidotti, A.: Benzodiazepine and GABA interactions: Role of GABA-modulin. In Usdin, E., Skolnick, P., Tallman, J.H., Greenblatt, D., and Paul, S. (Eds.): Pharmacology of Benzodiazepines, 1982, pp. 111-120.

Guidotti, A., Konkell, D.R., Ebstein, B., Corda, M.G., Krutzsch, H., Meek, J.L., and Costa, E.: Isolation, characterization and purification to homogeneity of GABA-modulin from rat brain. Proc. Natl. Acad. Sci. USA 79: 6085-6088, 1982.

Wise, B., Guidotti, A., and Costa, E.: Phosphorylation induces a decrease in the biological activity of the protein inhibitor (GABA-modulin) of γ -aminobutyric acid binding sites. Proc. Natl. Acad. Sci. USA 80: 886-890, 1983.

Corda, M.G., and Guidotti, A.: Modulation of GABA receptor binding by Ca^{++} . J. Neurochem., 1983, in press.

Costa, E., Corda, M.G., Epstein, B., Forchetti, C., and Guidotti, A.: GABA/benzodiazepine interaction in benzodiazepines: From molecular biology to the clinic. New York, Raven Press, in press, 1983.

Guidotti, A., Corda, M.G., Vaccarino, F.M., and Wise, B.C.: Role of GABA modulin, and of an endogenous effector of beta-carboline binding sites in the GABA/benzodiazepine receptor interaction. In Bowry, N. (Ed.): Action and Interaction of GABA and Benzodiazepines. In press, 1983.

Guidotti, A., Corda, M.G., Wise, B.C., Vaccarino, F., and Costa, E.: GABAergic synapses: Supramolecular organization and biochemical regulation. Neuropharmacology, in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01549-04 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of imipramine binding sites in rat brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M.L. Barbaccia Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 2.8	PROFESSIONAL: 2.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> To evaluate the role of high affinity ³H-imipramine binding sites located on the 5-HT axons in the down regulation of β-adrenergic receptors in CNS, a selective lesion of the 5-HT axon terminals obtained by an i.c.v. injection of 5,7-dihydroxytryptamine (5,7-DHT) was carried out. This 5,7-DHT lesion prevents the loss of β-adrenergic receptor recognition sites and the attenuation of the isoproterenol-sensitive adenylate cyclase activity or of the cAMP accumulation measured in cortical membranes or minces of rats treated repeatedly with imipramine or desipramine. These results suggest that a neuronal loop connecting 5-HT axons with NE synapses participates in the down regulation of NE-receptor function and perhaps in the antidepressant action of imipramine and its congeners. The V_{max} of 5-HT uptake by hippocampal minces is increased when the number of ³H-imipramine binding sites to hippocampal membranes is decreased after chronic imipramine and desipramine. Also the inhibitory effect on the 5-HT uptake by various imipramine concentrations "in vitro" is attenuated. Thus an endogenous effector of the ³H-imipramine binding sites may play a physiological role in the regulation of 5-HT uptake. We have partially purified a thermostable nonpeptidic endogenous effector of imipramine binding sites from rat brain which inhibits 5-HT uptake and displaces ³H-imipramine binding. Evidence is available that this endogenous effector is not 5-HT or tryptamine. This effector may be of importance as a biochemical marker to study the action of imipramine and the etiology of certain types of depression. Crude synaptic membranes of rat brain also contain specific high affinity binding sites for an atypical antidepressant, mianserin. The number of ³H-mianserin binding sites is increased following 5,7-DHT lesion, whereas the density of 5-HT₂ recognition sites labelled by ³H-ketanserin is unchanged. Repeated daily injections with imipramine decrease the number of ketanserin binding sites but not of mianserin binding sites. Thus the mianserin recognition site appears to be a modulatory site distinct from 5-HT₂ recognition sites, though both contribute to the function of 5-HT receptor complex. Studies have been undertaken in order to elucidate the molecular mechanisms underlying the β-receptor down-regulation elicited by repeated treatments with the other atypical antidepressants such as iprindole and bupropion. </p>		

Project Description:

This project was done in collaboration with O. Gandolfi, Guest Worker; D.M. Chuang, Chemist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Though antidepressant drugs have been used extensively in the treatment of depression, neither the molecular mechanisms of the drug action nor the etiology of the disease is well understood. Recently, high affinity binding sites specific for typical antidepressants such as imipramine and desipramine and atypical antidepressants such as mianserin have been reported to be present in various brain structures of several species. These discoveries have provided a new tool to study the molecular events involved in the therapeutic effects of antidepressant drugs. Various investigators have suggested that desensitization of β -adrenergic receptors (or decrease in NE-sensitive adenylate cyclase) in brain of rats after chronic treatment with antidepressants is most likely linked to their therapeutic action. Selective lesion of 5-HT axon terminals were carried out by injecting i.c.v. 5,7-dihydroxytryptamine (5,7-DHT). To evaluate the role of imipramine binding sites, which have been shown by us and others to be present on 5-HT axon terminals, on the down-regulation of NE-sensitive adenylate cyclase induced by protracted imipramine or desipramine treatment, we have found that this 5,7-DHT lesion prevents the loss of β -adrenergic recognition sites as well as the attenuation of the responsiveness of adenylate cyclase to isoproterenol in isolated cortical membranes of rats chronically treated with desipramine. Moreover the attenuation of the NE-sensitive cAMP generating system in cortical minces induced by imipramine is also prevented by lesion of 5-HT nerve terminals. In contrast, down-regulation of the NE-stimulated cAMP accumulation in cortical slices elicited by repeated administrations with mianserin is unaffected by 5,7-DHT lesion. These results suggest that a neuronal regulatory loop might connect 5-HT terminals with the neurons where β -adrenergic receptors are located and that this link participates in the attenuation of NE-receptor function and, perhaps, in the antidepressant action of imipramine and related drugs.

Several lines of evidence indicate that imipramine binding sites are related to a regulatory site of 5-HT uptake system. We have found that the B_{max} of 3H -imipramine binding to crude synaptic membranes prepared from hippocampi of rats receiving imipramine (twice daily for 3 weeks) is reduced whereas the net uptake of 5-HT (V_{max}) by hippocampal minces is increased. Also the inhibitory effect on the 5-HT uptake by various imipramine concentrations added "in vitro" to the hippocampal minces is attenuated when the number of 3H -imipramine binding sites is decreased by repeated imipramine injections. Our data support the possibility that the sites where 3H -imipramine binds play a physiological role for the regulation of 5-HT uptake. Hence 5-HT uptake system functions as a supramolecular entity where various subunits are involved in the fine tuning of the uptake process. In support of these inferences we have partially purified a thermostable nonpeptidic endogenous effector of 3H -imipramine binding sites from rat brain which, in a dose dependent manner, inhibits 5-HT uptake and displaces 3H -imipramine binding. We have tested this endogenous modulator on 3H -flunitrazepam, 3H -mianserin, 3H -dihydroalprenolol binding and none of these ligands could be displaced by the inhibitor of 3H -imipramine binding. We can exclude that this biological activity is due to 5-HT or tryptamine because during the chromatographic procedures of the putative endogenous effector, these two molecules are retained in the conditions in which the putative effector is eluted. Preliminary results suggest that this effector might not contain carboxylic group but contain an amino and hydroxyl group. This effector may be of importance as a biochemical marker to study the action of imipramine and to study the molecular nature of the biochemical defect operative in certain types of depression.

Crude synaptic membranes of rat brain contain specific high affinity binding sites for an atypical antidepressant, mianserin. It was previously suggested that ^3H -mianserin labels 5-HT_2 receptor recognition sites because this binding can be effectively displaced by spiperidol and ketanserin (J. Pharmacol. Exp. Ther. 216:142-148 (1981)). However we have shown that the number of ^3H -mianserin binding sites are increased following lesion of 5-HT axons with 5,7-dihydroxytryptamine whereas the binding characteristics of ^3H -ketanserin remain unchanged. Moreover repeated daily injections of imipramine decrease the specific binding of ^3H -ketanserin but fail to affect the binding of ^3H -mianserin to crude synaptic membranes prepared from rat hippocampus or cortex. These results suggest that the binding sites for mianserin and 5-HT_2 recognition sites are not identical but they may interact allosterically. A working hypothesis is that 5-HT axons produce 2 chemical signals, each one of them acting on a different synapse. One is serotonergic, the other has 2 specific recognition sites, one for the signal produced by the 5-HT axon that acts through the ^3H -mianserin binding site; the other labeled by ^3H -ketanserin or ^3H -spiperidol which is called 5-HT_2 receptor and is modulated by the effector produced by 5-HT axons that binds on ^3H -mianserin binding site. We are currently trying to verify this model by studying the interactions between the ^3H -ketanserin and the ^3H -mianserin binding sites and by trying to isolate the possible endogenous effector for the ^3H -mianserin site.

We have also studied the mechanisms of action of two other atypical antidepressants, iprindole and bupropion. Repeated daily injections of iprindole for 21 days decrease the density of β -adrenergic receptor binding sites and NE-sensitive adenylate cyclase activity in the frontal cortex. However these iprindole-induced events are unaffected by a lesion of 5-HT axon terminals. Long term but not acute administrations with iprindole decrease the number of ^3H -ketanserin and ^3H -mianserin binding sites in the frontal cortex and hippocampus but do not modify the binding characteristics of ^3H - 5-HT_1 receptor recognition sites. Experiments are in progress to determine whether these iprindole effects on ^3H -ketanserin and ^3H -mianserin binding require the function of intact 5-HT axon terminals. Bupropion was considered to be an atypical antidepressant because it was reported by others that this drug upon chronic treatments fail to modify the function of β -adrenergic receptors. However we found that in the brain of rats treated with relatively high doses of bupropion (50 mg/kg, twice daily) for 3 weeks, the density of β -adrenergic receptor recognition sites and the activity of NE-sensitive adenylate cyclase are both attenuated. The specificity of this bupropion effect is supported by the finding that the binding of ^3H -mianserin, ^3H -ketanserin and ^3H - 5-HT to crude synaptic membranes is unaffected following long term bupropion administration. Currently we are studying the molecular mechanisms of the bupropion elicited down-regulation of β -adrenergic receptor function.

The present study has moved an important step toward the understanding of the etiology of mental depression and the therapeutic action of several antidepressant drugs. The endogenous ligands for imipramine and mianserin binding sites in CNS may be casually related to the disease state of certain forms of mental depression and their levels in the cerebral spinal fluid may therefore be used as a biochemical marker of depression. Further purification and characterization of these endogenous ligands and the search for other classes of endogenous ligands are now in progress. These studies could lead to formulation for a better therapy of affective disorders.

Publications:

Barbaccia, M.L., Brunello, N., Chuang, D.M., and Costa, E.: On the mode of action of imipramine: Relationship between serotonergic axon terminal function and down-regulation of beta-adrenergic receptors. Neuropharmacology 22: 373-383, 1983.

Barbaccia, M.L., Gandolfi, O., Chuang, D.M., and Costa, E.: Differences in the regulatory adaption of the brain recognition sites labeled by ^3H -mianserin or ^3H -ketanserin. Neuropharmacology 22: 123-126, 1983.

Barbaccia, M.L., Brunello, N., Chuang, D.M., and Costa, E.: Serotonin elicited amplification of adenylate cyclase activity in hippocampal membrane from adult rat. J. Neurochem. 40: 1671-1679, 1983.

Barbaccia, M.L., Chuang, D.M., Gandolfi, O., and Costa, E.: Transsynaptic mechanisms in the action of imipramine. In Usdin, E., and Stephanis, C. (Eds.): Frontiers in Neuropsychiatric Research, in press.

Costa, E., Chuang, D.M., Barbaccia, M.L., and Gandolfi, O.: Molecular mechanisms in the action of imipramine. Experientia, in press.

Barbaccia, M.L., Gandolfi, O., Chuang, D.M., and Costa, E.: Modulation of neuronal 5-HT uptake by a putative endogenous ligand of imipramine recognition sites. Proc. Natl. Acad. Sci. USA, in press.

Gandolfi, O., Barbaccia, M.L., Chuang, D.M., and Costa, E.: Daily bupropion injections for 3 weeks attenuate the NE-stimulation of adenylate cyclase and the number of β -adrenergic recognition sites in rat frontal cortex. Neuropharmacology, in press.

Costa, E., Barbaccia, M.L., Gandolfi, O., and Chuang, D.M.: Endogenous modulation of serotonin uptake as a site for the action of imipramine. In Biggio, G., and Costa, E. (Eds.): Advances in Biochemical Psychopharmacology. New York, Raven Press, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01550-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biochemical mechanisms regulated by various receptors in anterior pituitary		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation)		
P. Onali	Visiting Associate	SMRP NIMH
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 2.3	PROFESSIONAL: 1.5	OTHER: 0.8
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The interaction of stimulatory and inhibitory receptors at the level of <u>adenylate cyclase</u> has been studied in three systems. In rat <u>anterior pituitary</u> , <u>vasoactive intestinal peptide</u> (VIP) stimulates <u>adenylate cyclase</u> and <u>prolactin</u> release in the <u>mammotrophs</u> . <u>Dopamine</u> can block both of these responses through action at a D-2 receptor. <u>Cholera toxin</u> also activates <u>adenylate cyclase</u> and DA can block this effect by acting on a D-2 receptor. The rat <u>pituitary GH3 cell line</u> provides a single population of cells which also respond to VIP with both <u>adenylate cyclase</u> activation and <u>prolactin</u> secretion. <u>Muscarinic agonists</u> can inhibit both basal and VIP-stimulated <u>adenylate cyclase</u> , as well as <u>prolactin</u> secretion, in this cell line. Changes in <u>cyclase</u> activity correlate with changes in <u>cyclic AMP</u> content and in <u>prolactin</u> release, suggesting that <u>cyclic AMP</u> serves as one second messenger for regulation of <u>PRL</u> secretion. <u>Muscarinic</u> receptors in rat <u>striatum</u> also inhibit <u>adenylate cyclase</u> and concurrently stimulate a high affinity <u>GTPase</u> , suggesting that <u>inhibitory</u> receptors may affect <u>adenylate cyclase</u> via action on a <u>GTPase</u> . The <u>GH3</u> cells will allow us to examine this question further in a pure cell population.		

This project was done in collaboration with M. Olinas, Visiting Associate; Carola Eva, Guest Worker; J.P. Schwartz, Research Chemist; S. Lofstrandh, Technician and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Neurotransmitters may modify adenylate cyclase by either stimulating or inhibiting its activity. In a given cell, opposing regulatory inputs may converge on this enzyme system and regulate the rate of formation of cyclic AMP. Thus the identification of these modulators and the study of their mechanism of action constitute a crucial step in the understanding of the transmembrane regulation of adenylate cyclase. These studies have utilized three tissues, rat anterior pituitary, the rat pituitary GH3 cell line, and rat striatum.

Dopamine modulates the adenylate cyclase of rat anterior pituitary by decreasing the response of this enzyme to the stimulatory effect of vasoactive intestinal peptide (VIP), a prolactin-releasing hormone. This original observation led us to investigate the mode of interaction of the dopamine receptors with the adenylate cyclase system of the anterior pituitary. By using primary cultures of rat anterior pituitary, we observed that dopamine was able to inhibit the adenylate cyclase activity stimulated by either VIP or cholera toxin, specific activators of the enzyme and effective prolactin-releasing factors. The inhibitory effect of dopamine was concentration-dependent, required the presence of guanine nucleotides and was blocked by specific dopaminergic antagonists. Because of the GTP dependency and of the inability of dopamine to inhibit the activation of adenylate cyclase by agents which act directly on the catalytic subunit of the enzyme, we concluded that the inhibitory coupling of the dopamine receptors with the adenylate cyclase occurred at the level of the regulatory protein(s) of this enzyme system. One of the functional properties of these proteins is the ability to bind and to hydrolyze GTP by a specific high affinity GTPase. Therefore, the activity of these GTPases could be an expression of the level of involvement of the regulatory proteins in the response of the enzyme system to neurotransmitters.

In rat striatum, the occupancy of muscarinic receptors inhibits the adenylate cyclase activity present in synaptic plasma membranes. These membranes contain a high affinity GTPase activity, which can be detected under the same experimental conditions used for the measurements of adenylate cyclase activity. Therefore we have investigated the effect of the activation of the muscarinic receptors on this GTPase activity and compared these effects with the action of these receptors on the adenylate cyclase. We have found that acetylcholine stimulated the GTPase activity with an apparent EC_{50} of 3 μ M, similar to that found for the inhibition of adenylate cyclase. Acetylcholine increased the V_{max} of the GTPase, without changing the K_m for GTP. The rank order of potency of various cholinergic agonists to stimulate GTPase correlates with their ability to inhibit adenylate cyclase. Atropine, but not d-tubocurarine, antagonized the stimulation of GTPase by acetylcholine. The hydrolysis of GTP was competitively inhibited by a stable analog of GTP, GMP-PNHP, which does not substitute for GTP in supporting the inhibitory effect of acetylcholine on the adenylate cyclase activity. Treatment of the membranes with cholera toxin does not affect the ACh-stimulated GTPase but amplifies the extent of adenylate cyclase inhibition elicited by ACh. These results indicate that the activation of GTPase is associated with the inhibitory coupling of the muscarinic receptors to the adenylate cyclase system. We are currently investigating the effect of agents known to modify the a.c. activity on the hydrolysis of GTP.

The rat pituitary tumor GH3 cell line, which secretes prolactin and growth hormone, has receptors for muscarinic agonists and for the vasoactive intestinal peptide. ACh inhibits prolactin release while VIP stimulates it. We have found that ACh inhibits both basal and VIP-stimulated adenylate cyclase activity, at concentrations comparable to those which affect prolactin secretion. The ACh effect is mediated through a muscarinic receptor; it can be mimicked by other muscarinic agonists and blocked by atropine. In intact cells, ACh decreases cyclic AMP content in parallel with its effects on PRL release, whether the cells have been incubated with or without VIP. The inhibitory effect of ACh on adenylate cyclase thus correlates with its inhibitory effect on cyclic AMP and on PRL release. We are currently investigating interactions between VIP and TRH, another peptide which increases PRL release but is thought not to act via cyclic AMP, as well as between ACh and somatostatin, a peptide which inhibits PRL release.

These studies are of interest not only for elucidating the role of cyclic AMP in the regulation of release of pituitary hormones but also for the demonstration of interactions between different neurotransmitters in the regulation of cell function. They allow us to study the biochemical mechanisms underlying these interactions and to study the mechanism of action of several peptide transmitters.

Publications:

Onali, P., Schwartz, J.P., and Costa, E.: Inhibition of VIP-sensitive adenylate cyclase by dopamine in rat anterior pituitary. In Jacob, J., Kuriyama, K., Segawa, T., and Yamamura, H.J. (Eds.): Molecular Pharmacology of Neurotransmitter Receptor Systems. New York, Raven Press, 1983, pp. 199-207.

Onali, P., Schwartz, J.P., and Costa, E.: Inhibitory coupling of dopamine receptors to adenylate cyclase in rat anterior pituitary. In Biggio, G., Costa, E., Gessa, G., and Spano, P.F. (Eds.): Receptors as Supramolecular Entities. England, Pergamon Press, in press.

Onali, P., Eva, C., Olanas, M.C., Schwartz, J.P., and Costa, E.: In GH3 pituitary cells acetylcholine and vasoactive intestinal peptide (VIP) antagonistically modulate adenylate cyclase, cyclic AMP content and prolactin secretion. Mol. Pharmacol., in press.

Onali, P., Olanas, M.C., Schwartz, J.P., and Costa, E.: Involvement of a high affinity GTPase in the inhibitory coupling of striatal muscarinic receptors to adenylate cyclase. Mol. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01551-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Is insulin a neuromodulator in the central nervous system?		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) D.M. Chuang, Chemist, SMRP, NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.60	PROFESSIONAL: 0.60	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Rat brain contains insulin-like peptide and binding sites recognized by insulin. Using olfactory bulb slices, we have found that cAMP content can be increased by either insulin or dopamine (DA). The insulin-dependent accumulation of cAMP was facilitated by sulpiride but was unaffected by haloperidol. Simultaneous addition of insulin and DA failed to increase cAMP when GppNHp was present, indicating that insulin interacts with DA and that insulin may be a neuromodulator for DA. We are now studying the synaptic location and transcriptional regulation of mRNA's for insulin-like peptide using a cDNA probe prepared from a bacterial clone synthesizing rat proinsulin. Since internalization of insulin may be a mechanism that explains the interactions between DA and insulin in rat brain, we have also studied insulin internalization using frog erythrocytes as a model system. Using erythrocytes incubated with ¹²⁵I-insulin, we have found a temperature-dependent and energy required accumulation of radioactivity in the intracellular function. Morphologically we were also able to visualize insulin internalization using cells incubated with rhodamine-labeled with insulin. </p>		

Proposed Course:

This work was done in collaboration with M. L. Barbaccia, Visiting Fellow, P. Panula, Visiting Fellow and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

This project has been terminated.

Publications:

Barbaccia, M.L., Chuang, D.M. and Costa, E.: Is Insulin a Neuromodulator? In: Costa, E. and Trabucchi, M. (eds.): Advances in Biochemical Psychopharmacology, Volume 33: New York, Raven Press, pp. 511-518, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01552-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Agonist and antagonist of benzodiazepine receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M. Ferrari Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) Stimulation of benzodiazepines by various ligands can elicit opposite types of responses such as <u>proconvulsant</u> and <u>anticonvulsant</u> or <u>anxiogenic</u> and <u>anxiolytic</u> . In order to answer whether a new class of drugs belong to <u>benzodiazepine</u> (anxiolytic), <u>beta-carboline</u> (anxiogenic) or <u>RO 15-1788</u> (antagonist) type of ligand we have developed a behavioral animal model that predicts the anxiogenic and anxiolytic potency of a drug.		

Project Description:

This project was done in collaboration with M.G. Corda, Guest Worker, A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

For these experiments we used the behavioral paradigm developed by Vogel. Water-deprived rats were placed in the experimental chamber where they had access to water through a stainless steel drinking tube extending 2 cm into the chamber. In absence of aversive stimulus, animals licked without interruption for the testing period (3 min) totaling approximately 28-30 drinking periods (one drinking period is equal to 3 sec of cumulative drinkometer output). A conflict situation which suppresses responding was induced by the application of an electric shock at the end of each 3 sec drinking period. A current intensity of 1 mA suppressed the number of drinking periods. Diazepam and other anxiolytic benzodiazepines reduced in a dose-dependent manner the behavioral inhibition induced by punishment.

Since one of our goals was to develop a test to evaluate a proconflict action and to rank the drugs eliciting various intensities of proconflict effect, the intensity of the aversive stimulus (electric shock) was reduced in order to find the intensity that would leave almost unchanged spontaneous water drinking and hence allow drug-induced conflict to be easily observed. We noticed that if the intensity of the electric shock was reduced from 1 mA to 0.20-0.30 mA the paradigm proposed by Vogel became suitable to evaluate drugs which have a proconflict action.

Among the drugs studied was FG 7142 (beta-carboline-3-carboxylic acid ethylester methylamide) which is anxiogenic in man, and other beta-carboline derivatives. All these drugs elicited a proconflict action, while RO 15-1788 and CGS 8216 (2-phenylpyrazolo[4,3]-quinolin-3-[5H]one) antagonize the proconflict action of FG 7142 or that of other beta-carbolines. The proconflict response induced by beta-carboline derivatives was elicited by doses which are significantly lower than those reducing unpunished drinking and are not due to change in thirst or pain threshold or to electrical seizures of selected brain structures that could be detected only through an EEG study. These findings suggest that chemicals that act as ligands of benzodiazepine receptors can be classified into three categories:

1. Ligands which possess anticonflict action. These diazepam-like drugs are ineffective in the proconflict test but they block the proconflict action of beta-carboline derivatives.
2. Ligands which possess proconflict action. These drugs, such as the beta-carboline derivative FG 7142, have no effect in the anticonflict test but block the effect of diazepam.
3. Ligands which possess neither proconflict nor anticonflict actions. These drugs (RO 15-1788 and CGS 8216) are, however, able to block the anticonflict and proconflict actions of benzodiazepine and beta-carboline derivatives, respectively.

Isoniazid, a drug which decreases GABAergic transmission by blocking GABA synthesis, potentiates the action of beta-carboline derivatives in the proconflict test. Moreover, pentylenetetrazol, a drug that selectively antagonizes the GABA-mediated post synaptic inhibition of blocking Cl^- conductance (McDonald and Barker, 1978), acts as a potent

proconflict agent. This suggests that the behavioral actions of beta-carbolines may be mediated by a down regulation of GABA receptor function.

The importance of the present observation is that these proconflict and anticonflict tests can be used to further investigate whether drug-induced shifts in conflict behavior are correlated with functional interactions between the various structural components of GABA receptors, and whether the activity of drug in this test correlate with anxiogenic or anxiolytic potency in man.

This behavioral test can be a powerful tool in identifying benzodiazepine ligands with potential anxiolytic, anxiogenic or antagonistic activity. This test has also been used for studying the behavioral effects of endogenous ligand for benzodiazepines.

If the effect of drugs on this animal behavioral test should relate to their clinical efficacy as anxiolytic or anxiogenic agents, the test will help to elucidate pathological anxiety and etiology of convulsive diseases and to predict possible therapeutic applications of agonists and antagonists of benzodiazepine receptors.

We intend to study on this model test a series of benzodiazepine agonist and antagonist ligands and correlate their potency in this test with their potency in other pharmacological and clinical tests. We also want to use this test to study the role of GABA in anxiety and to screen for possible endogenous effectors of the benzodiazepine recognition sites.

Publication:

Corda, M.G., Blaker, W.D., Mendelson, W.B., Guidotti, A., and Costa, E.: Beta-carbolines enhance shock-induced suppression of drinking in rats. Proc. Natl. Acad. Sci. USA 80: 2072-2076, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01553-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Biosynthesis of enkephalins in bovine adrenal medulla		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) I. Lindberg Staff Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.1	PROFESSIONAL: 1.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This work represents a continuation of our investigation into the biosynthetic mechanisms responsible for the production of met-enkephalin in adrenal medullary chromaffin granules. We have purified a trypsin-like adrenomedullary protease capable of generating met-enkephalin from endogenous precursors 1000 fold using affinity chromatography in combination with gel filtration. The enzyme has an apparent molecular weight of 20,000 by gel filtration. The reactivity of the adrenal enzyme toward fluorogenic substrates and enkephalin-containing peptides was studied. The results indicate that the fluorogenic compounds are poor substrates for the enzyme; however, Peptides E and F (endogenous adrenal opioids each containing two enkephalin sequences) were cleaved. When the enzymatic products of these peptides were identified by HPLC, it was found that cleavage occurred between or after pairs of basic residues. The 8.6 Kdal N-terminal fragment of proenkephalin (which contains a carboxyl terminal met-enkephalin) and [³H]-lipotropin were also used as substrates for this enzyme. While the 8.6 Kdal fragment was cleaved to form met-enkephalin and lys-met-enkephalin, no cleavage of [³H]-lipotropin was detectable. We have recently begun biosynthetic studies on the octapeptide met-enk-arg-gly-leu⁸. We have developed a sensitive radioimmunoassay for this peptide, and examined its distribution and molecular heterogeneity in adrenal and brain tissue. The brain distribution of the octapeptide was found to closely parallel the distribution of met-enkephalin; however, in several brain areas, octopeptide immunoreactivity was heterogeneous with respect to molecular weight. Chromaffin granules contained mainly high molecular weight forms of met-enk-arg-gly-leu⁸. Whether these high molecular weight forms function as precursors for octapeptide formation will be examined in future studies. </p>		

Project Description:

This project was done in collaboration with H.-Y.T. Yang, Pharmacologist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

The adrenal medulla is a rich storehouse of variously sized met-enkephalin-containing peptides; it is thought that some of the high molecular weight forms of enkephalin in adrenal medulla may represent precursors to met⁵- and leu⁵-enkephalin. We have examined bovine adrenal chromaffin granules for the presence of enzymes capable of generating met⁵-enkephalin from endogenous precursors. We have previously reported the partial purification of a serine protease from chromaffin granules. In the past year we have extended these studies by further purifying the adrenal protease and examining its reactivity toward several substrates. The substrates chosen were: 1) small synthetic fluorogenic peptides containing basic residues; 2) the heptapeptides met⁵-enk-arg⁶-arg⁷- and met⁵-enk-arg⁶-phe⁷; 3) Peptides E and F, adrenal peptides with enkephalin sequences at amino and carboxyl termini; 4) an 8.6 Kdal fragment of proenkephalin containing met-enkephalin at its carboxyl terminus and 5) [³H]-lipotropin. It was found that the Km of the enzyme toward the best fluorogenic substrate, Boc-glu-lys-lys-MCA, was in the millimolar range, while the Km of the enzyme toward the 8.6 Kdal proenkephalin fragment was approximately 0.5 M. The two heptapeptides and [³H]-lipotropin proved to be poor substrates for the enzyme; however, Peptides F and E were cleaved. The kinetics of the hydrolysis of Peptides E and F are presently under investigation. The site of cleavage of the various substrates was studied by subjecting the enzymatic products to HPLC. It was found that cleavage of Peptide F yielded arg⁶-met⁵-enkephalin, met⁵-enkephalin and lys⁶-met⁵-enkephalin, while cleavage of Peptide E yielded leu⁵-enkephalin and arg⁶-arg⁷-met⁵-enkephalin. The 8.6 Kdal fragment of proenkephalin was hydrolyzed to form met⁵-enkephalin and lys⁶-met⁵-enkephalin. We are currently attempting to use ion-exchange HPLC to purify the adrenal enzyme to homogeneity, and hope to use immunohistochemical means to verify that this enzyme is indeed present in chromaffin granules. Other plans include the use of chromaffin cell cultures as an in vitro model to explore the effects of protease inhibitors on the biosynthesis of enkephalin.

We have recently initiated studies on the processing of proenkephalin to the octapeptide met⁵-enk-arg⁶-gly⁷-leu⁸. A highly sensitive antiserum to the octapeptide was developed, and the distribution of this peptide was examined in brain and adrenal tissue. The distribution of the octapeptide in brain was found to closely parallel the distribution of met⁵-enkephalin, providing evidence that in the brain, as in the adrenal, both are derived from the same proenkephalin precursor. When the immunoreactivity present in brain regions was characterized by gel filtration, it was found that certain brain regions (such as the medulla-pons and the hypothalamus) contained considerable quantities of high molecular weight material, corresponding to an apparent molecular weight of ~8,000 daltons. In acid extracts prepared from chromaffin granules, more than 80% of octapeptide immunoreactivity eluted on Sephadex G-75 as this 8-10,000 dalton species. Whether the high molecular weight immunoreactive material found in the brain is identical to that found in the adrenal is currently under investigation.

Significance to Biomedical Research

The present investigation is clearly relevant to biomedical research in that it represents an attempt to understand the enzymatic mechanisms responsible for the biosynthesis of an important neuropeptide, met⁵-enkephalin. Once the enzymatic processes by which the

enkephalins are synthesized are clearly identified, it will become possible to study the regulation of enkephalin biosynthesis through an examination of the effects of pharmacologic manipulation of enkephalin-generating enzymes.

Publication:

Lindberg, I., Yang, H.-Y.T., and Costa, E.: Characterization of a partially purified trypsin-like enkephalin-generating enzyme in bovine adrenal medulla. Life Sci. 31: 1713-1716, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01555-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Enkephalin metabolism		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J. Chou Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> It has been of interest to determine the specific enzymes involved in the <u>catabolism</u> of endogenous <u>opioid peptides</u> and then, in turn, to develop selective inhibitors for these enzymes. Research on this aspect has been mostly on enkephalins but the metabolism of other endogenous opioid peptide is less well explored. Previously we have observed in vitro that <u>met-enkephalin-arg-phe</u> (ME-arg-phe) can be readily hydrolyzed by a <u>dipeptidyl carboxypeptidase</u> and, furthermore, this reaction can be effectively inhibited by <u>captopril</u>. In this study, whether the dipeptidyl carboxypeptidase participates in physiological inactivation of ME-arg-phe released was investigated using the in vivo superfusion of rat spinal cord. The release of opioid peptides, from spinal cord was induced by substance P. Without peptidase inhibitors, the efflux of ME-arg-phe and met-enkephalin (ME) was hardly detectable. The inclusion of captopril in the perfusion medium increased the recovery of released ME-arg-phe but not that of ME. Combination of captopril and <u>bestatin</u> further increased ME-arg-phe release slightly. The recovery of ME released was greatly enhanced by bestatin but not by captopril or <u>thiorphan</u> (generally known as enkephalinase inhibitor). The results suggest that the dipeptidyl carboxypeptidase may play an important role in inactivation of released ME-arg-phe but not that of ME in vivo. Furthermore, the analgesic effect of ME-arg-phe applied intrathecally was greatly potentiated by captopril. Therefore it is highly probable that the dipeptidyl carboxypeptidase participate actively in the termination of ME-arg-phe activity in the spinal cord. The catabolism of ME released from spinal cord seems to be actively metabolized by aminopeptidase. </p>		

Project Description:

This project was done in collaboration with H.-Y.T. Yang, Pharmacologist and J. Tang, Visiting Fellow, SMRP, NIMH.

Since the discovery of the endogenous opioid peptides, it has been of interest to determine what happens to them after their release. Most research on catabolism of opioid peptide has been concentrated on enkephalins and inactivation of other opioid peptide is less well known. In this project, inactivation of opioid heptapeptide, met⁵-enkephalin-arg⁶-phe⁷ (ME-arg-phe), was studied. Previously we have found, *in vitro*, that ME-arg-phe can be readily hydrolyzed to met⁵-enkephalin (ME) and arg-phe by a dipeptidyl carboxypeptidase which can be effectively inhibited by captopril. Using captopril as a tool, physiological relevance of this reaction, whether it participate in inactivation of ME-arg-phe or in formation of ME by using ME-arg-phe as precursor, was investigated.

Specifically, the effect of captopril and other peptidase inhibitor on the recovery of ME-arg-phe and ME release elicited by substance P from rat spinal cord was studied. The "in vivo" superfusion of rat spinal cord through indwelling cannulae by a push-pull pump as described by Yaksh et al. (J. Neurophysiol. 46:1056-1075, 1981) was used. In this preparation, ME-arg-phe as well as ME were released when the spinal cord was superfused with substance P (10^{-6} M) containing Krebs's solution, but the amount of these release as detected in the superfusate is very low. However when appropriate peptidase inhibitor was included in the perfusion medium, the recovery of opioid peptides released into the superfusate was increased: 1) captopril increased the amount of released ME-arg-phe greatly but that of ME only very slightly, 2) bestatin enhanced markedly the amount of released ME but only slightly that of ME-arg-phe, 3) thiorphan, an enkephalinase inhibitor, exerted only a very weak and no protective effect on the released ME and ME-arg-phe respectively, 4) combination of bestatin and captopril increased the amount of released ME-arg-phe and also that of ME greatly. The results suggest that the dipeptidyl carboxypeptidase, which can be effectively inhibited by captopril, may play an important role in physiological inactivation of released ME-arg-phe in spinal cord. However, the results fail to support a role of the dipeptidyl carboxypeptidase in formation of met⁵-enkephalin by using ME-arg-phe as precursor.

ME-arg-phe applied intrathecally showed a transient and weak activity in increasing the tail flick latency of the rat tail exposed to radiant heat. This effect of ME-arg-phe was enhanced and prolonged when rats were pretreated intrathecally with combination of bestatin and captopril. This result further prompted us to evaluate the pharmacological usefulness of captopril at spinal cord level. The effect of intrathecally applied captopril on the tail flick latency of rats treated with electro-acupuncture was studied. Electro-acupuncture, at 3V for 10 min, increased the tail flick latency of rats significantly and this effect was further potentiated by the intrathecal application of captopril.

The biological significance of this study is that inhibitors of ME-arg-phe inactivating enzyme may be designed and then in turn used as a pharmacological tool in further exploring the functional role of the opioid heptapeptide, ME-arg-phe, which is widely distributed both in CNS and peripheral tissues.

The proposed course of this study is 1) to determine the physiological relevance of the dipeptidyl carboxypeptidase in ME-arg-phe catabolism in the brain, 2) to continue searching for efficient inhibitors which are capable of penetrating blood brain barrier and 3) to study the effect of captopril on catabolism of other endogenous opioid peptide such as met⁵-enkephalin-arg-gly-leu and dynorphin(1-8).

Publications:

Yang, H.-Y.T., Harsing, L.G., Majane, E.M., and Costa, E.: Possible role of dipeptidyl carboxypeptidase in metabolism of met⁵-enkephalin and met⁵-enkephalin-arg⁶-phe⁷. In Costa, E., and Trabucchi, M. (Eds.): Advances in Biochemical Psychopharmacology, Vol. 33. New York, Raven Press, 1982, pp. 209-215.

Zhang, A.-Z., Tang, J., Chou, J., Yang, H.-Y.T., and Costa, E.: Met⁵-enkephalin-arg⁶-phe⁷: Neurochemical and neuropharmacological considerations. In Degradation of Endogenous Opioids: Its Relevance in Human Pathology and Therapy. New York, Raven Press, 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01556-03 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Release of endorphins		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) L. Harsing Visiting Associate SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.4	PROFESSIONAL: 0.4	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> In this study, the mechanism involved in the anorectic effect of d-fenfluramine and CM 57 277 was investigated. Repeated injections of these two anorectic drugs resulted in an elevation of hypothalamic met⁵-, leu⁵- and beta-endorphin. This increase is associated with a reduction in body weight. The effect of these two anorectics can be reversed by metergoline, a serotonin receptor antagonist. The results suggest that a decreased utilization of hypothalamic opioid peptides caused by a facilitation of serotonergic transmission may be responsible for the anorectic action of fenfluramine and CM 57 277 but not that of amphetamine. Met⁵-enkephalin release was also investigated in this study. The interaction between serotonergic and the endogenous opioid systems and the importance of endogenous opioid peptides in eating behavior are well demonstrated in this study. These findings may sharpen our understanding of normal eating behavior. The interactions of met⁵-enkephalin, other transmitters, and substance P, was also investigated in this study. Met⁵-enkephalin can be released from periaqueductal gray slices by substance P suggesting that substance P analgesia may be mediated by opioid peptides. </p>		

Project Description:

This work was performed in collaboration with H.-Y.T. Yang, Pharmacologist; E. Costa, Chief of the Laboratory of Preclinical Pharmacology and J. Del Rio, Guest Worker, SMRP, NIMH.

This project has been terminated.

Publications:

Harsing, L.G., Yang, H.-Y.T. and Costa, E.: γ Evidence for a GABA mediation in the benzodiazepine inhibition of the release of met γ -enkephalin elicited by depolarization. J. Pharmacol. Exp. Ther. 223: 689-694, 1982

Del Rio, J., Naranjo, J.R., Yang, H.-Y.T. and Costa, E.: Substance P-induced release of met γ -enkephalin from striatal periaqueductal gray slices. Brain Research, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01558-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Immunohistochemical studies on neurotransmitters in the nervous system		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) P. Panula Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Pharmacodynamics		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 2.0	PROFESSIONAL: 2.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Bombesin/gastrin releasing peptide-like immunoreactivity</u> existed in two molecular weight forms in rat hypothalamus, spinal cord and sensory ganglia. In the hypothalamus, this immunoreactivity was found in areas which did not contain substance P-immunoreactive cells. <u>Met-enkephalin-arg-gly-leu-like immunoreactivity</u> was widely distributed in rat brain and spinal cord. In the brain, this immunoreactivity was found in the same locations with other opioid peptides derived from preproenkephalin A. <u>Motilin-like immunoreactivity</u> was found in the arcuate nucleus of the hypothalamus. Using antisera against <u>histamine</u>, the brain histamine-containing neuronal system was revealed. The cell bodies were located in the basal hypothalamus around the premammillary nuclei and in the caudal magnocellular nucleus. </p>		

Project Description:

This project was done in collaboration with Dano B. Cosenza-Murphy, Research Biologist and H.-Y.T. Yang, Pharmacologist, SMRP, NIMH.

The aim of this study was to localize new neurotransmitters and neuropeptides in the central and peripheral nervous system to reveal the interrelationships of these neuronal systems with previously characterized pathways and target organs.

Bombesin/gastrin releasing peptide-like immunoreactivity (BN/GRP-LI) and substance P-like immunoreactivity (SP-LI) were found in different locations in the rat hypothalamus, whereas both immunoreactivities were found in the dorsolateral tegmental nucleus in the hindbrain. Gel filtration chromatography revealed two molecular weight forms of BN/GRP-LI in the hypothalamus, spinal cord and sensory ganglia. Neither of these was identical to synthetic bombesin. The high molecular weight form coeluted with synthetic porcine GRP. Thus, it is possible that authentic bombesin does not exist in mammalian CNS but the immunoreactivity detected represents different fragments of GRP.

An opioid peptide, met⁵-enkephalin-arg⁶-gly⁷-leu⁸, was localized in rat tissues using immunocytochemical methods. Nerve cell bodies were found in different brain areas including the piriform cortex, olfactory tubercle, caudate nucleus, lateral septum, numerous hypothalamic nuclei, central grey and several hindbrain nuclei.⁵ In most areas, this immunoreactivity was colocalized with another opioid peptide, met⁵-enkephalin-arg⁶-phe⁷. The results indicate that the brain enkephalin precursor molecule contains both of these opioid peptides and thus resembles the precursor found in the adrenal gland. In the spinal cord, met⁵-enkephalin-arg⁶-gly⁷-leu⁸-like immunoreactivity was concentrated in laminae I and II of the posterior horn, where dense networks of terminals were found. Nerve cell bodies with this immunoreactivity were found in deeper parts of the posterior horn. Only nerve fibers and terminals exhibited this immunoreactivity in the anterior horn. Immunoreactive nerve fibers and terminals in the posterior pituitary, myenteric plexus and adrenal gland were also immunoreactive. Some chromaffin cells in the adrenal gland and SIF cells in sympathetic ganglia exhibited this immunoreactivity. In human sympathetic ganglia, only nerve fibers with this immunoreactivity were detected. Nerve fibers and terminals in human sympathetic ganglia also exhibited substance P-, bombesin/GRP-, GRP(1-16)- and met⁵-enkephalin-arg⁶-phe⁷-like immunoreactivities. The results show that the peptides stored in rat and human sympathetic ganglia show marked similarity.

Immunohistochemical studies with antibodies against motilin showed that cell bodies storing this peptide are located in the basal hypothalamus, mainly in the arcuate nucleus. The specificity of this reaction was established using blocking controls. In contrast, only a weak reaction which was not blocked by excess of peptide was observed in the cerebellum. The results indicate that the motilin-like immunoreactivity previously reported in the cerebellum may be different from the motilin-like immunoreactivity in the hypothalamus.

A specific antiserum against histamine was produced in rabbits. This antiserum revealed histamine-like immunoreactivity in the mast cells, enterochromaffin-like cells in the stomach, and neurons in the basal hypothalamus. The specificity of the reaction was established by extensive blocking controls and solid phase immunoabsorption. In the hypothalamus, nerve cells were found at the level of premammillary nuclei. Numerous cells in the caudal magnocellular nucleus exhibited histamine-like immunoreactivity. Histamine-immunoreactive nerve cells were not found in the mesencephalon and pons-medulla, though immunoreactive fibers were seen. This indicates that the basal hypothalamic nuclei

may provide the whole brain with a widespread projection of histamine-containing fibers. The studies are now aimed at the possible coexistence of other neuroactive substances with histamine in these cells and the relationship of the brain histamine neurons with other neuronal systems.

The substances included in this study act as neurotransmitters or neuromodulators in the central and peripheral nervous system and are involved in mediation of pain sensation from the periphery through the spinal cord to higher centers of the brain. They are also involved in thermoregulation, control of the autonomic nervous system and hormone secretion. The exact localization of these peptides is a necessary step for a better understanding of the neuronal mechanisms which regulate these physiological functions and disorders of the sensory systems and endocrine organ. Future research will involve molecular biological studies on the regulation of the function of the described neuronal systems.

Publications:

Panula, P., Yang, H.-Y.T., and Costa, E.: Neuronal location of the bombesin-like immunoreactivity in the central nervous system of the rat. Regul. Peptides 4: 275-283, 1982.

Panula, P., Yang, H.-Y.T., and Costa, E.: Coexistence of met⁵-enkephalin-arg⁶-phe⁷ with met⁵-enkephalin and the possible role of met⁵-enkephalin in neuronal function. In Palay, S., and Chan-Palay (Eds.): Coexistence of Neuroactive Substances in Neurons. In press.

Panula, P., Hadjiconstantinou, M., Yang, H.-Y.T., and Costa, E.: Immunohistochemical localization of bombesin/GRP and substance P in primary sensory neurons. J. Neurosci., in press.

Panula, P., Cosenza-Murphy, D., Yang, H.-Y.T., and Costa, E.: Binding of GRP(14-27) but not bombesin or GRP(1-27) to hypothalamic magnocellular elements: An immunohistochemical study. J. Histochem. Cytochem., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01559-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Met ⁵ -enkephalin-arg ⁶ -phe in the brain and spinal cord		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) H.-Y.T. Yang Pharmacologist SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Previously, a radioimmunoassay method was developed to study the distribution of met⁵-enkephalin-arg⁶-phe (ME-arg-phe) in the brain and this opioid heptapeptide was found to be most concentrated in striatum. In this study, the interaction between ME-arg-phe and dopamine in striatum was studied. Chronic treatment of rats with haloperidol elevated the ME-arg-phe content in striatum but not in other regions. Following inhibition of the ME-arg-phe degradation by captopril, the accumulation rate of ME-arg-phe was greater in haloperidol treated rats than in control rats. The results suggest that haloperidol, a dopamine receptor blocker, increases ME-arg-phe biosynthesis. Previously, it was found that chronic haloperidol treatment also increased the content of met⁵-enkephalin (ME). In view of the fact that ME and ME-arg-phe are derived from the same precursor, preproenkephalin A, it is suggested that chronic haloperidol treatment enhances the preproenkephalin A biosynthesis. Cardioexcitatory neuropeptide, phe-met-arg-phe-NH₂ (FMRF-NH₂) was originally isolated from ganglia of macrocallista mimbosa and subsequently existence of FMRF-NH₂-like immunoreactivity in rat CNS was reported. Because of its structural similarity to ME-arg-phe, the distribution and biological activity of the FMRF-NH₂ immunoreactive material in CNS was studied. In rat brain and spinal cord, FMRF-NH₂-like immunoreactivity is unevenly distributed. The highest content is in hypothalamus and the lowest in cerebellum. In spinal cord, FMRF-NH₂ immunoreactivity is higher in dorsal horn. Biochemical analysis of the FMRF-NH₂ like material revealed that endogenous FMRF-NH₂ like material is similar to but not identical to authentic FMRF-NH₂. Because of this, endogenous FMRF-NH₂ material was partially purified and biological property tested. The endogenous FMRF-NH₂ like material was found to reduce the analgesic effect of ME-arg-phe when injected intrathecally. The function of FMRF-NH₂ like material will be further studied. </p>		

Project Description:

This project was done in collaboration with E.M. Majane, Chemist; J. Tang, Visiting Fellow; P. Panula, Visiting Fellow; J. Chou, Visiting Fellow; M.I. Iadarola, PRAT Fellow and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Previously, the distribution and characteristics of the opioid heptapeptide, met⁵-enkephalin-arg⁶-phe⁷ (ME-arg-phe), in the rat brain was studied. During the course of this study, phe-met-arg-phe-NH₂ (FMRF-NH₂)-like immunoreactivity in rat central nervous system was described by immunohistochemical technique (Weber et al., Science 214:1248, 1981). Interestingly, in the FMRF-NH₂ bioassay system using organs from molluscs, ME-arg⁶-phe⁷ was found often to have opposite effects of FMRF-NH₂. Because of the close relationship between these two neuropeptides, we have decided also to study, in rat spinal cord and brain, the distribution, biochemical characterization and biological activity of FMRF-NH₂ like peptide.

Antiserum to FMRF-NH₂ was prepared by immunizing rabbits with FMRF-NH₂ hemocyanin conjugate. The antiserum shows no significant affinity for cholecystokinin (CCK)8, CCK4, substance P, met⁵-enkephalin but cross reacts with ME-arg-phe very slightly (<0.1%). The FMRF-NH₂-like immunoreactivity is unevenly distributed in rat brain: the highest concentration is in hypothalamus and the lowest in cerebellum. Similarly to the results of immunohistochemical study (Weber et al., Science 214:1248, 1981), the distribution of FMRF-NH₂ like immunoreactivity in the brain differs from that of ME-arg-phe. In the spinal cord, FMRF-NH₂-like immunoreactivity is higher in dorsal half (0.46±0.02) than in ventral half (0.18±0.01).² Rostro-caudally, FMRF-NH₂-like immunoreactivity is evenly distributed ranging from 0.21±0.01 to 0.29±0.02 for cervical to sacral region. For comparison, the distribution of ME-arg-phe immunoreactivity in rat spinal cord was also studied. Unlike FMRF-NH₂ immunoreactivity, the content of ME-arg-phe is higher in the caudal than in the rostral regions of the spinal cord. Dorsro-ventrally, the ME-arg-phe immunoreactivity is higher in dorsal half than in ventral half, the highest concentration is in the dorsal gray and the lowest in the ventral white.

The cellular location of FMRF-NH₂ and ME-arg-phe immunoreactivity was studied by immunohistochemical technique. ME-arg-phe like immunoreactivity was found mainly in nerve terminal-like structure in lamina I and II. Deeper layer of the dorsal horn exhibited occasional immunoreactivity. FMRF-NH₂-like immunoreactivity was found only in lamina I of the dorsal horn and the density of immunoreactive terminal was low. In the ventral horn, ME-arg-phe was distributed in terminal-like structure and varicose fiber throughout the gray matter whereas no FMRF-NH₂ like immunoreactivity was found in ventral horn. Both immunoreactivities were found in a dense plexus of varicose fibers in layer X around the central canal.

The FMRF-NH₂-like immunoreactive material was analyzed by gel filtration and HPLC. The results indicate that the main endogenous FMRF-like peptide is larger than the authentic FMRF-NH₂ in molecular weight and is not reactive to ME-arg-phe antiserum. The immunoreactivity of FMRF-NH₂-like material, similarly to authentic FMRF-NH₂, is reduced by CNBr, almost totally abolished by trypsin, but not affected by carboxypeptidase A treatment. The results indicate that the endogenous FMRF-NH₂ like material is similar to but not identical to authentic FMRF-NH₂. Because of this, endogenous FMRF-NH₂ immunoreactive material was partially purified by antibody sepharose affinity column chromatography from bovine medulla oblongata and biological property tested. When

injected intrathecally, the endogenous FMRF-NH₂ like material was found to reduce the analgesia effect of ME-arg-phe.

Previously we found that chronic treatment of rats with haloperidol increases the met⁵-enkephalin content of rat striatum. Both met⁵-enkephalin and ME-arg-phe are derived from the same precursor, preproenkephalin A. Because of this, whether haloperidol also increases the striatal content of ME-arg-phe was studied. ME-arg-phe content of rat striatum increased in dose and time dependent manners three weeks after a daily intraperitoneal injection of haloperidol. The action of haloperidol occurred selectively in dopamine-rich brain area. Following intraventricular captopril, the accumulation rate of ME-arg-phe (30%) was greater in haloperidol than in saline injected rats (14%). Captopril (0.5 mg, intraventricular injection) inhibits the ME-arg-phe degradation, the greater accumulation rate of ME-arg-phe suggests that haloperidol increases the polypeptide biosynthesis. A slower rate of ME-arg-phe release in haloperidol treated rats can be excluded as a cause for the drug-induced increase in content because the peptide is released by a similar concentration of K⁺ in striatal slices of haloperidol and saline treated rats. The similarities between the accumulation of ME-arg-phe and of met⁵-enkephalin induced by haloperidol can be used to infer that haloperidol increases the biosynthesis of the specific mRNA for preproenkephalin, an opioid peptide precursor, which contains one copy of ME-arg-phe and several copies of met⁵-enkephalin.

The significance of this study on biomedical research is that the antagonistic effect of FMRF-NH₂ like material may serve as a tool to explore the function of opioid peptide.

The proposed course of this study is to purify the endogenous FMRF-NH₂ like material and determine its 1) structure and 2) biological activity especially its interaction with opioid including ME-arg-phe.

Publications:

Yang, H.-Y.T., Panula, P.,⁷ Tang, J., and Costa, E.: Characterization and location of [met⁵]-enkephalin-arg⁶-phe⁷ stored in various rat brain regions. J. Neurochem. 40: 969-976, 1983.

Majane, E.A., Iadarola, M.I., and Yang, H.-Y.T.: Distribution of met⁵-enkephalin-arg⁶-phe⁷ in rat spinal cord. Brain Res. 264: 336-339, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01560-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Met ⁵ -enkephalin-arg ⁶ -phe in peripheral tissue		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J. Tang Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.7	PROFESSIONAL: 0.7	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The presence of <u>neuropeptides</u> as well as <u>peptide hormones</u> in <u>lung</u> has been well established. Previously we have detected <u>met⁵-enkephalin-arg⁶-phe</u> (ME-arg-phe), bombesin and <u>substance P-like peptides</u> in the rat lung by specific radioimmunoassays. In this study, the cellular locations of these peptides were investigated by immunohistochemical technique. Bombesin and substance P immunoactivities were found in nerve fibers present in bronchi and blood vessels while ME-arg-phe immunoactivity was localized in small APUD-like cells around the wall of some bronchi. Because of its cellular location, the release of ME-arg-phe from lung slices was studied. Superfusion of lung cubes with Krebs' solution containing substance P or bombesin shows that ME-arg-phe can be released in a Ca⁺⁺ dependent manner. The results seem to suggest that when substance P and bombesin are released by nerve impulses from their storage site in nerve fibers, they can facilitate or even trigger the release of ME-arg-phe directly or indirectly. </p>		

Project Description:

This project was done in collaboration with J. Chou, Guest Worker; P. Panula, Visiting Fellow; H.-Y.T. Yang, Pharmacologist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

We have previously detected met⁵-enkephalin-arg⁶-phe⁷ (ME-arg-phe) like immunore-active peptide in the extract of rat, guinea pig and human lung. Immunohistochemically this opioid peptide was found to be stored in APUD-like cells closely associated with the wall of small and medium size bronchilli.

In this study, in addition to this opioid peptide, the existence of other neuropeptide, substance P and bombesin like peptide, was also detected in the lung. The biochemical analysis of these two peptides by BioGel column chromatography followed by HPLC showed that substance P immunoreactivity is composed mainly of authentic substance P and bombesin immunoreactivity is consisted of multiple forms, which includes significant amount of high molecular weight material. ME-arg-phe, substance P and low molecular weight bombesin like peptide were measured after their separation by HPLC and were found to be 0.68 ± 0.08 , 1.3 ± 0.14 and 1.76 ± 0.12 pmol/mg protein respectively. Immunohistochemically, substance P like immunoreactivity was found in varicose fibers in the lung parenchyma, in loose connective tissue around the bronchi, in blood vessel walls and in bronchial walls. Bombesin-like immunoreactivity was located in nerve fibers. The bombesin and substance P positive cell bodies were not detected.

Because of their cellular locations, the effect of substance P and bombesin on the release of ME-arg-phe from APUD-like cells was studied by superfusion of rat lung cubes in vitro. Addition of substance P (10^{-6} M) or bombesin (10^{-7} M) in the perfusion medium was found to significantly increase the efflux of ME-arg-phe above the basal level. This ME-arg-phe efflux elicited by substance P or bombesin was abolished when Ca^{++} was omitted from the perfusion medium.

Several lines of evidence suggest the possible involvement of endogenous opioid peptide in respiration (review by Olson et al., Peptide 3:1039-1072, 1982). The significance of this study to biochemical research is that the effect of opioid peptide on respiration may now be investigated at the level of lung in addition to at the level of central nervous system.

The proposed course of this study is 1) to further search for the presence of other family of opioid peptide in the lung and 2) to further study the interaction of opioid peptides with other transmitters.

Publications:

Tang, J., Yang, H.-Y.T., and Costa, E.: Distribution of met⁵-enkephalin-arg⁶-phe⁷ (MEAP) in various tissues of rats and guinea pigs. Life Sci. 31: 23-3-2306, 1982.

Tang, J., Yang, H.-Y.T., and Costa, E.: Distribution of met⁵-enkephalin-arg⁶-phe⁷ in various tissues of rats and guinea pigs. Neuropharmacology 21: 595-597, 1982.

Tang, J., Chou, J., Zhang, A.Z., Yang, H.-Y.T., and Costa, E.: Met⁵-enkephalin-arg⁶-phe⁷ and its receptor in lung. Life Sci. 32: 2371-2377, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01561-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Cholecystokinin in brain		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M.J. Iadarola Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This research has centered around <u>interrelationships between cholecystokinin-containing neurons and opioid peptide-containing neurons in brain and spinal cord</u>. More precisely it represents the prelude to studying the relationship. At present, we are in the process of <u>quantitating</u> (via specific radioimmunoassay), <u>localizing</u> (via immunocytochemistry) and <u>characterizing</u> (via HPLC and gel filtration) these peptides in various systems. One system of particular interest is the mesolimbic dopamine system in which CCK coexists with dopamine. These neurons may interact with enkephalin and dynorphin neurons in nucleus accumbens - olfactory tubercle and possibly in striatum. These studies are of particular relevance to schizophrenia in which a disorder of dopamine transmission is hypothesized especially in the limbic and cortical projections which also contain CCK. In spinal cord CCK may act to influence our preception of tactile and thermal stimuli especially painful stimuli. CCK also acts to antagonize morphine analgesia. We are presently characterizing opiate peptides in cord representative of the pro-enkephalin A and B families (e.g. dynorphin 1-17, dynorphin 1-8, met-enkephalin, met-enkephalin-arg⁸-phe⁹ and met-enkephalin-arg⁶-gly⁷-leu⁸) as well as CCK. The studies on spinal cord are important to our understanding of pain, its pathways, the neurotransmitters involved, and the possibility of modulating morphine tolerance in chronic pain situations such as cancer. Lastly, we are in the initial stages of <u>cloning the CCK mRNA</u> in order to elucidate the structure of the CCK precursor molecule. This research will provide us with a new tool, called a <u>cDNA probe</u>, to investigate the <u>regulation of CCK</u> in brain by drugs, other transmitter systems, and behavioral manipulations. We hope also that this information will be useful to researchers and clinician working in <u>gastroenterology</u>, a field in which CCK is also very important. It seems likely that CCK not only helps us digest our food but helps us digest our thoughts as well! </p>		

Project Description:

This project was done in collaboration with H.-Y.T. Yang, Pharmacologist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Our previous work examined the co-localization of dopamine with cholecystokinin (CCK) in terminals of the mesolimbic DA system. Our studies indicated a topographic distribution of dopamine and CCK co-localization that conformed to the topographic distribution of the mesolimbic dopamine projections as opposed to the nigrostriatal dopamine projections. Attempts to characterize other CCK-containing pathways such as a hippocampal projection to accumbens or cortical projections to caudate have been negative. Application of immunocytochemical and retrograde anatomical tracing techniques is indicated; to this end we are engaged in raising antibodies to CCK suitable for immunocytochemistry. We have also examined post mortem human brain tissue from schizophrenics, normals and other psychiatric diagnosis for changes in CCK content. We have also developed a technique for labeling CCK in brain *in vivo*. We have applied this technique to estimate turnover and have tried various drugs and lesions to perturb the CCK system. These studies are in progress. An interaction between CCK and opioid peptides has been described for analgesia and morphine tolerance. We have begun to fully characterize the CCK and opioid system in spinal cord for members of the pro-enkephalin A family (met⁵-enkephalin, met⁵-enkephalin-arg⁶-phe⁷ and met⁵-enkephalin-arg⁶-gly⁷-leu⁸) and the pro-enkephalin B family (dynorphin 1-17, dynorphin 1-8). We have developed an RIA for dynorphin 1-17 and are in the process of developing one for dynorphin 1-8. Morphine tolerance may involve tonic release of CCK. It is therefore possible that we can use this system to study CCK turnover with our *in vivo* labeling method.

In addition we embarked upon a new effort designed to elucidate the structure of the CCK precursor molecule. We shall be employing molecular cloning and DNA sequencing techniques to decode the mRNA for CCK and thereby obtain the primary protein sequence. This should greatly enhance our understanding of the biochemistry of this system and our ability to probe the workings of CCK neurons in brain. We have begun this process with an oligonucleotide probe complementary to the base sequence for the CCK mRNA and have sequenced this probe with the Maxam-Gilbert technique in order to ensure its identity. The cloning experiments are extremely important: so much additional knowledge has been gained by cloning and sequencing the mRNA precursor for the opiate peptides that we feel it worthwhile to sequence the precursor for CCK.

Publications:

Iadarola, M.J., and Yang, H.-Y.T.: Relationship between cholecystokinin in rat forebrain: a biochemical study of co-localization. J. Neurochem., submitted.

Kleinman, J.E., Iadarola, M.J., Govoni, S., Hong, J., Gillin, J.C. and Wyatt, R.J.: Post-mortem measurements of neuropeptides in human brain. Psychopharmacol. Bull., submitted.

Meek, J.L., Iadarola, M.J., and Giorgi, O.: Cholecystokinin turnover in brain. Brain Res., in press.

Majane, E.M., Iadarola, M.J., and Yang, H.-Y.T.: Distribution of met⁵-enkephalin-arg⁶-phe⁷ in rat spinal cord. Brain Res., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

ZO1 MH 01562-02 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

The cholinergic neuronal system

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

P.E. Potter

Visiting Fellow

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

2.0

2.0

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects

☐ (b) Human tissues

☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to identify and study the acetylcholine-containing neurons. Our present objective is to study spinal cord cholinergic neurons and to develop a simple procedure for evaluating the rate of formation of acetylcholine.

Project Description:

This project was done in collaboration with M. Hadjiconstantinou, Guest Worker and N.H. Neff, Chief of Section on Biochemical Pharmacology, SMRP, NIMH.

We have developed a simple method for assaying choline and acetylcholine in neuronal tissue (Z01 MH 01562-01 SMRP). Our present goal was: 1) to study cholinergic neurons of spinal cord; and 2) to determine whether our analytical procedure might be used to study the rate of formation of acetylcholine.

- I. We have measured the distribution of acetylcholine in the rat spinal cord and the consequence of cord transection on acetylcholine. Rats were killed by microwave irradiation to inactivate acetylcholine esterase. Acetylcholine was found to be rather evenly distributed throughout the cord with values ranging from about 200-300 pmol/mg prot. Two weeks after complete cord transection at the lower thoracic level acetylcholine content of cervical and lumbar cord was unchanged but there was about a 40 percent decrease in both dorsal white and dorsal gray of the thoracic cord. These results indicate that spinal cord contains intrinsic cholinergic neurons. The cholinergic system in cord contrasts with the catecholamine and serotonin nerves of cord which appear to originate in brain and descend into the cord. We have determined that some of the cholinergic neurons of spinal cord can be destroyed by administering 6-aminonicotinamide, an antimetabolite of nicotinamide.
- II. Our procedure for the analysis of acetylcholine involves an initial precipitation step followed by purification and separation of choline and acetylcholine from other substances by HPLC. After administering radioactive choline to mice we have determined by collecting the fractions that emerge from the analytical HPLC column that radioactivity is only associated with the choline and acetylcholine peaks.

Moreover, the acetylcholine peak and radioactivity are lost if samples are exposed to a high pH before injection into the HPLC system and the choline peak and radioactivity increase corresponding to the quantities lost from the acetylcholine peak. Thus, it would appear that our procedure might be used to estimate the formation of acetylcholine in vivo by following the change of specific radioactivity of choline and acetylcholine with time after injecting radioactive choline.

Acetylcholine was the first neurotransmitter to be described. Unfortunately it has not been investigated to the same extent as other transmitters because methods for its assay are either insensitive, complicated, require expensive reagents or require specialized equipment. Our analytical procedure surmounts these difficulties and thus should provide information about this neuronal system of brain. This is particularly important now that several brain disorders are associated with a deficiency of cholinergic nerves in brain i.e., Alzheimer's disease. Our goal is to provide new insight into these problems.

Future studies will be directed towards completing the method for determining acetylcholine turnover and utilizing the method to study brain function under various experiment conditions.

Publication:

Potter, P.E., Meek, J.L., and Neff, N.H.: Acetylcholine and choline in neuronal tissue measured by HPLC with electrochemical detection. J. Neurochem., in press.

NOTICE OF INTRAMURAL RESEARCH PROJECT

ZO1 MH 01563-02 SMRP

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)

Adenosine: A putative neurotransmitter

PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.)

(Name, title, laboratory, and institute affiliation)

W. Wojcik

PRAT Fellow

SMRP

NIMH

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Preclinical Pharmacology

SECTION

Biochemical Pharmacology

INSTITUTE AND LOCATION

NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032

TOTAL MANYEARS:

PROFESSIONAL:

OTHER:

1.1

1.1

0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects☐ (b) Human tissues☒ (c) Neither☐ (a1) Minors☐ (a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of this project is to identify and characterize neuronal systems that utilize adenosine as a transmitter or neuromodulator. Our present objective is to provide evidence that adenosine serves a role in neuronal function within the striatum and cerebellum and to determine if other putative neurotransmitter interact with the adenosine receptor.

Project Description:

This project was done in collaboration with D. Cavalla, Visiting Fellow and N.H. Neff, Chief of the Section on Biochemical Pharmacology, SMRP, NIMH.

Adenosine, a product of ATP metabolism, has many properties of a neurotransmitter. Adenosine, or analogues of adenosine, when administered to animals have biochemical, electrophysiological and behavioral actions which appear to be the result of interactions with one and/or two membrane receptors termed A_1 and A_2 adenosine receptors. Our objectives were: 1) to determine the apparent location of adenosine A_1 and A_2 receptors in striatum; 2) to determine if other neurotransmitter substances are negatively coupled to adenylate cyclase as is the adenosine A_1 receptor; and 3) to determine if adenosine A_1 receptors interact with other receptors that are negatively coupled to adenylate cyclase.

- I. Three separate lesions were performed on striatum to determine if A_1 or A_2 adenosine responsive adenylate cyclase were lost: injection of 6-hydroxydopamine into the medial forebrain bundle to destroy the dopaminergic pathways; injection of kainic acid directly into the striatum to lesion intrinsic neuronal cell bodies; and surgical decortication to remove the cortical-striatal pathways. We found A_2 receptors to be associated primarily with intrinsic striatal neurons while A_1 receptors were more diffuse with some associated with intrinsic neurons and some with cortical striatal neuronal terminals. We were unable to detect an association of A_1 or A_2 receptors with dopaminergic neurons.
- II. We have determined that GABA-B receptors are negatively coupled to adenylate cyclase of brain. GABA and (-)baclofen, a GABA analog, inhibited basal adenylate cyclase activity by 30-40 percent in homogenates of rat brain. Among the various brain regions studied, the striatum and cerebellum displayed the greatest responsiveness to baclofen. The inhibition of adenylate cyclase was via the GABA-B receptor with agonist potencies being (-)baclofen GABA muscimol (+)baclofen. The inhibitory response to baclofen was insensitive to bicuculline, a specific GABA-A antagonist and was not affected by diazepam. From studies with neurologically mutant mice, an inhibitory response to baclofen was absent in animals that lacked granule cells.
- III. From studies with neurologically mutant mice we have determined that GABA-B and adenosine A_1 receptors are associated primarily with granule cells in cerebellum. There appears to be a biochemical interaction between these receptors for mediating inhibition of adenylate cyclase. By comparing dose-response curves for phenylisopropyladenosine (PIA), an adenosine receptor agonist, in the presence and absence of various concentrations of baclofen, a GABA-B receptor agonist, we observed that the curves for inhibition of adenylate cyclase were not parallel or additive, but that the same maximal inhibition occurred with high concentrations of either drug alone or in combination. The recognition sites for each receptor appeared to be independent when studied by ligand binding. The converging dose-response curves for baclofen and PIA indicate that the receptor recognition sites occur on the same membrane fragments derived from the same cells, cerebellar granule cells. They also indicate a possible common rate-limiting biochemical step in the mechanism by which adenosine A_1 and GABA-B receptors inhibit adenylate cyclase.

Some investigators have suggested that anxiolytic drugs act at adenosine receptor sites. At present, however, the role of adenosine in brain function is unclear. Our studies

are providing basic information needed to evaluate adenosine's role in brain physiology. They also demonstrate that endogenous substances that inhibit adenylate cyclase activity may act at specific receptors but may utilize the same post recognition site elements to reduce cyclase activity. Indeed, drugs acting at separate and specific receptors could produce the same pharmacological response in a tissue by utilizing the mechanism we have unmasked. Our studies of brain receptor mechanisms may provide a clue to the functional role of both adenosine and GABA-B neuronal systems.

Future studies will be directed towards isolating and characterizing the adenosine and GABA-B receptors of brain.

Publications:

Wojcik, W.J., and Neff, N.H.: Adenosine measurement by a rapid HPLC-fluorometric method: Induced changes of adenosine content in regions of rat brain. J. Neurochem. 39: 280-282, 1982.

Wojcik, W.J., and Neff, N.H.: Adenosine A_1 receptors are associated with cerebellar granule cells. J. Neurochem., in press.

Wojcik, W.J., and Neff, N.H.: Location of adenosine release and adenosine A_2 receptors to rat striatal neurons. Life Sci., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01564-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Control of GABA turnover in rat striatum		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) O. Giorgi Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Group on High Pressure Liquid Chromatography		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.1	PROFESSIONAL: 0.1	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) The object of this study was to examine the interaction of neurotransmitters in the striatum. GABA turnover was measured after local injection of neurotransmitter agonists and antagonists. This work, which was completed in the previous fiscal year was prepared for publication.		

Project Description and Proposed Course:

This project was done in collaboration with J.L. Meek, Pharmacologist, SMRP, NIMH.

The experiments performed previously for this project were summarized for publication and this project was terminated.

Publication:

Giorgi, O., and Meek, J.L.: GABA turnover in rat striatum: Effects of glutamate and kainic acid. J. Neurochem., in press, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01565-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Regulation of GABA_A and GABA_B receptor function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M.D. Majewska Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.3	PROFESSIONAL: 1.3	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> This study concerns an investigation of the role of <u>Ca²⁺</u> in the regulation of <u>GABA_A</u> binding to <u>GABA_A</u> and <u>GABA_B</u> recognition sites located in the synaptic membranes of <u>rat brain</u>. At 37° the binding of [³H]-GABA to GABA_B recognition sites is dramatically stimulated by Ca²⁺ with a <u>K_a ≈ 10⁻⁵ M</u> while the binding to GABA_A recognition sites is only slightly but significantly enhanced by Ca²⁺ with a <u>K_a ≈ 5 × 10⁻⁷ M</u>. The Ca²⁺ effect on GABA_A recognition sites is temperature dependent and requires <u>calmodulin</u> but involves neither phospholipase A₂ nor Ca²⁺-dependent proteases. Only GABA_A recognition sites are linked to <u>benzodiazepine</u> recognition sites and also this interaction is modulated by Ca²⁺ at physiological ion concentrations. Diazepam and low <u>μM Ca²⁺</u> cause the appearance of a high affinity binding site for GABA_A recognition sites. The number of GABA_B recognition sites measured at 37° is about 70% higher than that measured at 4°. This temperature-dependent increase in the number of GABA_B recognition sites is calmodulin independent, probably <u>Ca²⁺-dependent proteases</u> are operative. We have also studied whether GABA_A and benzodiazepines recognition sites located on <u>C6-glioma</u> and neuroblastoma NB2a cells are linked to some other transducer, for instance phospholipase A₂. In C6-glioma but not NB2a cells, prelabeled with [¹⁴C]-arachidonic acid, a GABA agonist muscimol stimulates the release of [¹⁴C]-arachidonic acid. This increased release is due to an activation of phospholipase A₂. This process is blocked by bicuculline, a classical GABA_A receptor blocker but not by inhibitors of Cl⁻ channel or GABA uptake blockers. The phospholipase A₂ activation by muscimol is potentiated by several benzodiazepines, but not by clonazepam. Analyses of the radioactive metabolites released by HPLC reveal that a substantial amount of <u>prostaglandin D₂</u> is formed when muscimol is supplemented with diazepam. Prostaglandin D₂ may be a neuromodulator which serves as a linkage of glial cells to neuronal function. We are currently investigating the possibility of the involvement of glial benzodiazepine receptors in the regulation of GABA function mediated through the formation of certain classes of prostaglandins. </p>		

Project Description:

This project was done in collaboration with D.M. Chuang, Chemist and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

The role of Ca^{2+} in the regulation of a variety of physiological processes is well recognized. Among others, Ca^{2+} has been shown to be required for the ligand binding to a novel class of GABA receptors discovered recently in rat brain synaptosomal membranes. This Ca^{2+} dependent GABA receptor can be labeled with (-)baclofen and has been termed GABA_B receptor recognition site in order to distinguish it from the classical GABA_A receptor recognition site which binds such ligands as bicuculline, THIP, muscimol or isoguvacine. We have investigated the mechanisms whereby Ca^{2+} regulates the binding of GABA to GABA_B recognition sites. We have also addressed the question of whether Ca^{2+} is involved in the regulation of GABA_A recognition sites and particularly in the interactions between receptor recognition sites for benzodiazepines and GABA .

At 37° the binding of $[^3\text{H}]\text{-GABA}$ to GABA_B recognition sites (measured in the presence of excessive amount of bicuculline or THIP) is stimulated by about 400% by Ca^{2+} . The K_a of Ca^{2+} for this activation is about 10^{-5}M . Among a spectrum of cations tested at 2.5 mM, only Ca^{2+} is able to increase greatly the binding to GABA_B sites. The Bmax of GABA_B binding measured in the presence of Ca^{2+} at 37° is about 70% higher than that at 4° . This temperature dependent effect is unrelated to calmodulin-mediated enzymatic processes such as phospholipase A_2 but may involve the action of Ca^{2+} -dependent proteases based on studies using various inhibitors of this proteases (iodoacetamide, hemin and leupeptin). Thus Ca^{2+} appears to play dual roles in the GABA binding to GABA_B recognition sites. This cation may participate in the regulation of the receptor number by an activation of the membrane-bound Ca^{2+} -protease(s) which by degradation of certain specific protein(s), may provide freedom to a latent pool of GABA_B recognition sites. On the other hand Ca^{2+} per se is also involved directly in the binding of GABA to this class of receptor recognition sites.

In contrast to GABA_B recognition sites, the binding of $[^3\text{H}]\text{-GABA}$ to GABA_A recognition sites (measured in the presence of excessive amount of (-)baclofen) is only slightly (by 25-30%) increased by relatively low concentration of Ca^{2+} . The K_a of Ca^{2+} for this activation is about $5 \times 10^{-7}\text{M}$. The Ca^{2+} effect on GABA_A recognition sites is also temperature dependent but does not involve Ca^{2+} -dependent proteases or phospholipase A_2 . Experiments using various inhibitors of calmodulin and synaptic membranes deprived of calmodulin suggest that this Ca^{2+} binding protein participates in the activation of GABA_A recognition sites in a Ca^{2+} dependent manner. Only GABA_A recognition sites are linked to benzodiazepine recognition sites and also this interaction is modulated by Ca^{2+} at physiological ion concentrations. Scatchard analyses revealed that in the presence of EGTA (free $\text{Ca}^{2+} < 10^{-8}\text{M}$) only one population of low affinity GABA_A binding site can be detected; however, when Ca^{2+} (μM) or diazepam are present in the system another high affinity binding site appears in addition to preexisting low affinity site. Regulation or modulation by Ca^{2+} of GABA binding to GABA_A and GABA_B receptor site occurs at physiological intracellular concentrations of this cation. This may suggest, that perhaps an increase of intracellular Ca^{2+} triggered by depolarization is responsible for the activation of homeostatic inhibitory mechanism in neurons, mediated through pre- and postsynaptic GABA receptors. This hypothesis is currently being tested.

Since both GABA_A and benzodiazepines recognition sites are located in the membranes of cultured C6-glioma and neuroblastoma NB2a, we have also investigated whether

these sites are linked with Cl^- channel or with some other transducer, for instance phospholipase A_2 . In C6-glioma but not NB2a cells, prelabeled with ^{14}C -arachidonic acid, a GABA receptor agonist muscimol stimulates the release of radioactive arachidonic acid into the medium. This increased release is associated with a reduction of a small pool of labeled phosphatidylcholine and phosphatidylethanolamine and is prevented by inhibitors of phospholipase A_2 including the Ca^{2+} -chelator EDTA. This process is not related to the GABA uptake system because it is insensitive to the uptake blockers beta-alanine and nipecotic acid. The muscimol-induced release of ^{14}C -arachidonate is blocked by bicuculline but is unaffected by picrotoxin and pentylenetetrazol, indicating that the effect is mediated by GABA recognition sites which are uncoupled from Cl^- channels. The phospholipase A_2 activation by muscimol is potentiated by flunitrazepam, midazolam, diazepam and medazepam (given in their potency order), but not by clonazepam. This benzodiazepine potentiation of muscimol effect is not antagonized by beta-carbolines, suggesting that a peripheral type of benzodiazepine recognition site is involved. Analyses of the radioactive arachidonate metabolites released in the medium by high pressure liquid chromatography revealed that muscimol alone stimulates the release of free arachidonic acid; however, when muscimol is accompanied by diazepam, a substantial amount of prostaglandin D_2 is released together with the free arachidonic acid. We propose that in glial cells possess a GABA-benzodiazepine receptor linked to mechanisms activating prostaglandin D_2 biosynthesis perhaps through an activation of a Ca^{2+} channel. A linkage of glial cells to neuronal function via prostaglandin D_2 will be proposed as a working model.

Prostaglandin D_2 is known to be an inhibitory neuromodulator and it can promote sleeping behavior when injected into rat brain. Thus the present finding has raised the interesting possibility of GABA-benzodiazepine receptor complex in glial cells participating in the sleep regulation and that the sleep-inducing properties of benzodiazepines are mediated at least in part through interactions with their glial receptors. We are currently testing this hypothesis by studying the interactions between neurons and glials in the expression of GABAergic function using a co-culture of both types of cells.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01566-02 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Molecular mechanisms in the antidepressant action of (-)deprenyl		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) G. Zsilla Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.5	PROFESSIONAL: 0.5	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) (-)Deprenyl is a selective inhibitor of monoamine oxidase (MAO) type B. MAO B uses as preferential substrates phenylethylamine and dopamine, while MAO A preferentially catabolizes norepinephrine (NE), dopamine and serotonin (5HT). At a dose of 5 mg per day, which selectively inhibits the MAO B, (-)deprenyl relieved many symptoms of depressed patients without eliciting the strong side-effects induced by other MAO inhibitors. In rats receiving (-)deprenyl (1 µmol/kg, s.c., for 21 days) the NE-stimulated cAMP accumulation and the β-adrenergic receptor number in minces and crude synaptic membranes from frontal cortex were significantly decreased. Moreover after the same treatment schedule the number of ³ H-imipramine recognition sites in frontal cortex and hippocampus was significantly increased. Interestingly, (-)deprenyl is not active "in vitro" as a displacer of ³ H-imipramine specifically bound to rat brain membranes and also the possibility that this effect is due to the formation of one of the (-)deprenyl metabolites, amphetamine, or to the blockade of MAO ₂ B was ruled out. A selective lesion of the 5HT axon terminals prevented the increase of ³ H-imipramine binding and the attenuation of the β-adrenergic-coupled cAMP generating system evinced by repeated injections of (-)deprenyl. Our results suggest that (-)deprenyl possesses many actions that are proper of antidepressants and by a primary modification of the serotonergic neurons can determine those modifications of the noradrenergic function that are believed to be beneficial to depressed patients.		

Project Description:

This project was done in collaboration with M.L. Barbaccia, Visiting Fellow; O. Gandolfi, Guest Worker and E. Costa, Chief of Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Since (-)deprenyl was claimed to be useful in the therapy of some forms of depression, we started to look for those neurochemical actions that could substantiate its use as an antidepressant. It is currently accepted that among the effects elicited in rat brain by repeated injections of various antidepressants those concerning the NE and 5HT receptor function are rather reproducible, with only a small number of partial exceptions. Typical and atypical antidepressants when given twice daily for two weeks or longer decrease the responsiveness of the cAMP generating systems linked to β -adrenergic receptor to NE stimulation. Many antidepressants decrease the number of binding sites for specific β -adrenergic receptor antagonists and the number of 5HT₂ recognition sites (labelled by ³H-spiroperidol or ³H-ketanserin) in frontal cortex and hippocampus of rat brain. In various animal species the brain membrane preparations contain specific recognition sites for typical and atypical antidepressants, namely imipramine and mianserin. A great proportion of the ³H-imipramine and ³H-mianserin recognition sites appear to be anatomically and functionally related to the 5HT system. Lesion studies have shown that ³H-mianserin binding sites are mainly located on cell bodies at the postsynaptic site of serotonergic synapses, while ³H-imipramine recognition sites are mainly located on 5HT axons and are functionally related to the 5HT uptake mechanism. Daily injections of imipramine or DMI-repeated for two or three weeks decrease the number of ³H-imipramine recognition sites. The latency time for this action is similar to that required for the down regulation of the β -adrenergic receptors and for the appearance of the therapeutic efficacy of the antidepressants. Rats were treated with (-)deprenyl following a close treatment schedule (1 μ mol/kg, s.c., for 21 days) in order to selectively block MAO type B. In these conditions the (-)deprenyl treatment induced a decrease of the NE stimulated cAMP accumulation in slices from rat frontal cortex and a decrease of the number of β -adrenergic recognition sites. The extent of this effect was comparable to that elicited by pargyline (20 mg/kg, i.p., for 21 days) which inhibits both MAO A and B. However unlike pargyline and many other antidepressants (-)deprenyl did not decrease the number of the recognition sites labeled by ³H-spiroperidol or ³H-ketanserin.

While the ³H-mianserin binding was unaffected after 3 weeks injections of (-)deprenyl it became apparent a significant increase of the number of ³H-imipramine recognition sites; this effect was present in frontal cortex and hippocampus but its extent was higher in the frontal cortex. A possible (-)deprenyl metabolite, amphetamine, injected for 3 weeks at a dose of 1 μ mol/kg, did not induce such an effect. Interestingly enough, a selective lesion of the 5HT axon terminals, obtained with the intracerebroventricular injection of 5,7-dihydroxytryptamine, completely abolished the enhancement of ³H-imipramine binding and the attenuation of the NE-stimulated cAMP accumulation induced by (-)deprenyl.

Our results indicate that daily injections of (-)deprenyl repeated for 3 weeks elicit a peculiar pattern of biochemical modifications that cannot be explained on the basis of the inhibition of MAO B; in fact pargyline either at low (selective for MAO B) or at higher doses (inhibits both MAO A and B) gave a completely different picture. Our present data suggest that the antidepressant action of (-)deprenyl could be due to the modification of the 5HT function. It has been reported that although 5HT is not a preferential substrate for MAO B, a high content of MAO B could be visualized by immunohistochemistry using a monoclonal antibody for MAO B in serotonergic neurons of rat brain. Our working hypothesis is that

(-)-deprenyl treatment affects a biosynthetic or catabolic pathway involved in the production or in the catabolism of a possible modulator of the 5HT function such as the modulator of the 5HT uptake that appear to operate through the interaction with the ^3H -imipramine recognition sites. If this turns out to be true it could represent a very important step ahead in the understanding of some of the mechanism that might be involved in the pathogenesis of affective disorders of man.

The identification of the molecular mechanism(s) whereby (-)-deprenyl increases the number of ^3H -imipramine recognition sites will be attempted. The possibility that (-)-deprenyl inhibits the release of the endogenous effector of imipramine recognition sites will be tested pending the identification of this effector.

Publication:

G. Zsilla, M.L. Barbacchia, O. Gandolfi, J. Knoll and E. Costa: (-)-Deprenyl a selective MAO "B" inhibitor increases ^3H -imipramine binding and decreases α -adrenergic receptor function. Eur. J. Pharmacol., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01567-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Role of synaptosomal basic proteins in the control of GABA receptor function		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) F. Vaccarino Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>GABA-modulin</u>, a basic protein which controls GABA benzodiazepine receptor interaction, was shown to be present in high concentration in association with GABA recognition sites in rat <u>brain synaptosomes</u>. <u>GM</u> is basic in nature and it is rich in arginine and lysine residues. The <u>above characteristics</u> link this peptide to the general class of <u>brain basic peptides</u>. However physiochemical and biochemical observations indicate that <u>GM</u> is <u>different from any other known basic peptide</u>. </p>		

Project Description:

This project was done in collaboration with A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

The binding of GABA to specific recognition sites on postsynaptic neuronal membranes is regulated by a peptide termed GABA-modulin (GM). GM purified from rat brain and added in vitro to crude synaptic membranes, noncompetitively reduces the number of high affinity GABA recognition sites. Hence, it appears to act as a coupling factor in the reciprocal interaction between recognition sites and other components of GABA/benzodiazepine receptor complex. Moreover, GM can be phosphorylated in vitro by cAMP-dependent protein kinase and this phosphorylation results in a loss of its inhibitory activity (Wise et al., PNAS 80: 886, 1983). In order to be functionally relevant the interaction between GM and GABA/benzodiazepine receptor observed in homogenates should be demonstrated at the synaptic level. To establish whether the location of GM is compatible with its proposed functional role, synaptosomal membranes were purified by the flotation-sedimentation density gradient centrifugation. These membranes contain high concentrations of GABA binding sites (B_{max} 5 pmol/mg protein) as well as GM (~ 10 μ g/mg protein). GM is a constituent of these membranes because it cannot be easily extracted by washing with iso-osmotic solutions but it is extracted with detergents. Treatment of synaptosomal membranes with 0.05% Triton X-100 results in a decrease of GM content and in a parallel 2-3 fold increase in 3H -GABA binding. GM extracted and purified from rat brain synaptosomes is composed of 129 amino acid residues, is rich in arginine and lysine residues; therefore the protein is basic. The above characteristics link this peptide to the general class of brain basic peptides. In particular synaptosomal GM is similar in structure to the small molecular weight myelin basic protein (RSBP); however, GM has a different amino acid composition, is higher in GLX (+7) and Lys (+5) residues and contains less Arg (-6) residues. Synaptosomal GM has a slightly different retention time on HPLC reverse phase C18 column using TFA/acetonitrile as eluting buffer. The tryptic map shows differences in four fragments between the two proteins. The migration of synaptosomal GM in SDS-PAGE and urea acidic gels is similar but not identical to that of the RSBP: the isoelectric focusing gel electrophoresis employing nonequilibrium pH gradient reveals a different chromatographic mobility: GM migrates toward the cathode as three major bands at a rate slower than that of RSBP. These results indicate that GM is an integral constituent of synaptosomal membranes and therefore may play a role in the regulation of GABAergic transmission and in the GABA/benzodiazepine receptor interaction. We believe that a more detailed study of the mode by which GM controls GABA receptor function may lead to new models to characterize potent and specific drugs which like benzodiazepines or the beta-carbolines may facilitate or inhibit GABAergic transmission.

Publication:

Vaccarino, F., Costa, E. and Guidotti, A. Synaptosomal basic proteins: differences from myelin basic proteins. Abs. Neurosci., 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER Z01 MH 01568-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Sulfacation of cholecystokinin and other brain peptides		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) <i>(Name, title, laboratory, and institute affiliation)</i> J.L. Meek Pharmacologist SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Group on High Pressure Liquid Chromatography		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.6	PROFESSIONAL: 1.6	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither </div> <div style="display: flex; justify-content: space-between; margin-top: 5px;"> <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Cholecystokinin octapeptide (CCK-8) occurs in relatively large amounts in some neurons of the cerebral cortex. Unlike most other mammalian neuropeptides, this compound contains a sulfate ester. We injected radiolabeled inorganic sulfate (³⁵S-sulfate) into rat cerebral cortex and measured the formation and disappearance of radiolabeled CCK-8 using HPLC. The data allow the first calculation of the turnover rate of a non-secretory neuropeptide in brain. The turnover of CCK-8 in cortex (half-life = 16 hr) is considerably slower than that of the biogenic amines and amino-acid neurotransmitters (half lives less than 4 hr). The enzyme responsible for sulfation is phenol sulfo transferase (PST). Since ability to inhibit sulfation would make possible a non isotopic method for CCK turnover, and aid in studying post-translational modification of proteins, we also examined the ability of the best available inhibitor of PST dichloronitrophenol to block sulfation in vivo of phenols with widely differing affinity for PST and to block incorporation of SO₄ into CCK-8.</p>		

Project Description:

This project was done in collaboration with O. Giorgi, Visiting Fellow and M.J. Iadarola, Guest Worker, SMRP, NIMH.

Although many proteins are sulfated during post-translational processing only a few active neuropeptides such as cholecystokinin (CCK-8) are known to contain sulfate. This fact has provided us with a new approach for measuring the turnover of a peptide. Previous attempts to study turnover (which used protein synthesis inhibitors or labeled amino acids) met with little success.

The object of this study was to determine whether CCK-8 turnover could be measured by following the formation and disappearance of ^{35}S -CCK-8 after local injection of $^{35}\text{SO}_4$.

The methodology used employed HPLC for separation of CCK-8 from other peptides, HPLC for measurement of inorganic sulfate, HPLC measurement of dichloronitrophenol, a phenol sulfotransferase inhibitor (PST), and a simple enzymatic assay of PST developed in this lab in 1972.

Major Findings:

- 1) ^{35}S -CCK-8 formation could be readily demonstrated in rat cortex after injection of ^{35}S - SO_4 .
- 2) The maximum radioactivity of CCK-8 occurred at 4 hr after injection, then radioactivity declined with a half life of 16 hr.
- 3) Free sulfate could be measured for calculation of specific activity by HPLC with indirect photometric detection using a mobile phase of disulfo dihydroxy benzene.
- 4) Dichloro nitro phenol inhibits brain phenolsulfotransferase with an IC_{50} of 12-14 μM for artificial substrates with Km 's which vary widely: p-nitro phenol (.3 μM) and dopamine (1,300 μM).
- 5) A dose of 100 $\mu\text{mol/kg}$ i.p. of dichloro nitro phenol gives a 25 μM brain concentration of the drug.
- 6) This dose of the drug blocks formation of ^{35}S -CCK-8 4 hr after injection of $^{35}\text{SO}_4$ by 60%.
- 7) The half-life of the inhibitor in brain and the toxicity (doses above 300 $\mu\text{mol/kg}$ were fatal) prevent the use of dichloro nitro phenol as a good inhibitor of PST.

Proposed Course:

Possible irreversible inhibitors of PST will be prepared by K. Kirk, NIAMD, and tested by us for ability to act *in vitro* and *in vivo*. If good inhibitors are found, studies of sulfation of proteins as a post translational step will be initiated. Studies of CCK-8 turnover will be made using a pharmacological approach.

Significance

Cholecystokinin is implicated in control of satiety, extrapyramidal function, pain perception and higher levels of brain function. Study of its turnover provides us with information on its alternation by drugs & disease states. In some brain areas, CCK is co-localized with dopamine. The turnover methods described here will permit study of whether drugs or lesions which affect dopamine turnover will induce parallel alterations in CCK-8 turnover.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01569-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Interaction with neuropeptides		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J. Tang Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Molecular Neurobiology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> Cholecystokinin-8-sulfate (CCK-8 sulfate) injected intrathecally antagonizes the morphine induced increase of tail flick latency in rats. The mechanism underlying this action of CCK-8-sulfate against morphine analgesia was investigated in this study. The CCK-8-sulfate antagonism of <u>morphine analgesia</u> was greatly reduced when rats were pretreated with proglumide, a cholecystokinin receptor antagonist. By using rats implanted with push-pull cannulae at spinal cord, the action of morphine on CCK release from spinal cord was tested. When subarachnoid spaces of the rat spinal cord was infused with morphine containing medium, efflux of CCK-like immunoreactive material was detected. This increased efflux of CCK-like material was reduced greatly by naloxone. The result seems to suggest that endogenous CCK may be important in development of morphine tolerance. Therefore, the effect of proglumide on development of morphine tolerance was tested. The tolerance to analgesia induced by repeated injections of morphine was markedly reduced when proglumide was given in combination with morphine to the rat. FMRF-NH₂ also antagonizes the tail flick latency increase elicited by morphine and this action is again antagonized by proglumide. Whether endogenous FMRF-NH₂ participates in development of <u>morphine tolerance</u> will be further investigated. </p>		

Project Description:

This project was done in collaboration with H.-Y.T. Yang, Pharmacologist; M.J. Iadarola, Guest Worker and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Cardioexcitatory neuropeptide phe-met-arg-phe-NH₂ (FMRF-NH₂) and cholecystokinin (CCK) are known to have some actions which are opposite those of the opiates (Greenberg et al., Fed. Proc. 42:82-86, 1983; Faris et al., Science 219:310-312, 1983). In searching for functional role of neuropeptide such as opioid peptides, FMRF-NH₂ and CCK, we have decided to explore the mechanism underlying the above observation.

CCK-8 suppresses analgesia induced by stress, morphine (Faris et al., Science 219:310-312, 1983) and β -endorphin (Stoh et al., Eur. J. Pharmacol. 80:421, 1982). We have also observed that CCK-8 (sulfate) injected intrathecally antagonizes the morphine induced analgesia as measured by tail flick latency. This effect of CCK-8 (sulfate) was antagonized by pretreatment of rats with proglumide, a blocker of CCK. In order to determine whether endogenous CCK participates in antagonizing the effect of morphine, release of CCK from spinal cord by morphine was studied by in vivo superfusion of the rat spinal cords through implanted cannulae by a push-pull pump with artificial CSF. Superfusion of subarachnoid space of the spinal cord with morphine (10^{-6} M) containing medium resulted in release of CCK immunoreactive material in the superfusate. This result suggests a role of CCK in morphine tolerance and thus led us to test the effect of proglumide on morphine tolerance. When rats were repeatedly injected every 2 hrs with morphine sulfate (10 mg/kg, s.c.), the animals were found to develop tolerance usually after 6th or 7th injection. However, if rats were treated with morphine and proglumide simultaneously, the morphine tolerance was either greatly reduced or not observed.

FMRF-NH₂ was also found to greatly reduce the morphine induced tail flick latency increase. Similarly to morphine, this effect is antagonized by proglumide. The result suggests that the effect of FMRF-NH₂ may be mediated through endogenous CCK. Whether endogenous FMRF-NH₂ play a role in morphine action is the proposed course for future study.

The significance of this study on the biomedical research is that the problem of morphine tolerance may be now better investigated based on this preliminary result.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01570-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) <u>Synthesis of the neurotransmitter pool of glutamate in the brain</u>		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) J.T. Wroblewski Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH <u>Laboratory of Preclinical Pharmacology</u>		
SECTION <u>Group on High Pressure Liquid Chromatography</u>		
INSTITUTE AND LOCATION <u>NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032</u>		
TOTAL MANYEARS: 0.8	PROFESSIONAL: 0.8	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p>Although <u>glutamate</u> is a major excitatory neurotransmitter in the CNS, the specific route of its synthesis is not known. A possible precursor is <u>ornithine</u>, which is known to be converted in vitro to glutamate via the enzyme <u>ornithine amino transferase (OAT)</u>. The aim of the present work was to determine whether <u>inhibition of OAT activity in vivo</u> by local injection of L-canaline would affect levels of glutamate, and whether such <u>changes</u> could be related to a specific glutamatergic pathway. A 100 µg dose of canaline decreased septal OAT activity by 90% in 5 min. Glutamate content decreased in 2 phases with half lines of 7 min and 8 hr. The rapidly turning over pool appears to be neuronal, since it was largely eliminated after lesion of the glutamatergic input to the septum. Studies are now under way of the incorporation of radiolabelled ornithine into glutamate in vivo. Initial experiments show that a rapid incorporation can be demonstrated.</p>		

Project Description:

This project was done in collaboration with W.D. Blaker, Staff Fellow, and J.L. Meek, Pharmacologist, SMRP, NIMH.

Glutamate is a major excitatory transmitter in the CNS, as well as serving important metabolic roles. Although there have been many studies to date of glutamate using iontophoresis, high affinity uptake, changes in content after lesion, and release of locally applied glutamate, there has been no way to study the dynamics of the system, i.e. turnover. It has not yet been established what the precursor of the neurotransmitter pool is. Earlier studies tested glucose and glutamine. Recent work has suggested ornithine may be converted by ornithine amino transferase (OAT) to glutamate. If this possibility is true, it will make possible new approaches to the study of this compound.

The objective of this study was to examine whether inhibition of OAT would affect levels of glutamate, and whether such changes could be related to a specific glutamatergic pathway.

The methodology used consisted of an automated HPLC apparatus for measurement of endogenous glutamate content and enzymatically formed glutamate for OAT assay. The potent irreversible OAT inhibitor canaline was injected into the septum of rats with or without lesions of the glutamatergic input from the hippocampus.

Major Findings

- 1) Canaline (100 µg intraseptally) inhibited OAT by 90% within 5 min.
- 2) After canaline, glutamate decreased in two phases, with half lives of 7 min and 8 hr.
- 3) Acute lesion of the fimbria (to stop firing of glutamatergic neurons) largely eliminated the rapidly turning over phase suggesting that ornithine may in fact serve as a precursor for neurotransmitter glutamate.
- 4) Chronic lesion of the fimbria did not alter septal OAT levels indicating that the enzyme is not exclusively contained in glutamatergic neurons.
- 5) Tracer amounts of ^3H -ornithine can be converted in vivo to glutamate when injected locally into the septum. This finding further supports the possibility that ornithine contributes in part to glutamate biosynthesis.

Proposed Course

- 1) The time course of conversion of intraseptally injected ^3H -ornithine into glutamate will be investigated.
- 2) The conversion of ^3H -ornithine into glutamate will be studied in rats with fimbria lesions.
- 3) If these studies are positive, a routine turnover method will be developed using intravenous or intraventricular injection of ^3H -ornithine.

Significance

As a major excitatory transmitter, glutamate may be involved in many disease states. As an example, in Alzheimers disease inputs to the substantia innominata may be affected. One likely input would be glutamate. It will be of acute interest to determine how this input can be pharmacologically altered.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01571-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) GABA/Benzodiazepine receptor complex in adrenal medulla		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) Y. Kataoka Visiting Fellow SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> <u>Adrenal chromaffin cells</u> contain high affinity saturable <u>benzodiazepine</u> and <u>GABA binding sites</u>. The pharmacological specificity and the interaction between these two binding sites is similar to that reported in brain. However, the benzodiazepine binding sites of adrenal medulla cells fail to bind beta-carbolines. Preliminary results suggest that diazepam and muscimol change the <u>release of enkephalin-like material</u> from primary culture of bovine adrenal medullary cells. </p>		

Project Description:

This project was done in collaboration with Y. Gutman, Visiting Scientist; A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

It has been reported that the behavioral effects of anxiolytic benzodiazepines or anxiogenic beta-carbolines are associated with concomitant peripheral effects due to central stimulation of adrenal medullary function. To elucidate whether these peripheral effects are the consequence of a direct action of benzodiazepine ligands on the adrenal medullary cells, we have examined if adrenal chromaffin cells contain receptors for benzodiazepines. Binding studies using ^3H -flunitrazepam indicate that this ligand binds to membranes obtained from cow adrenal medulla or from primary cultured cells with a K_d of approximately 10 nM and a B_{max} of approximately 80 fmol/mg prot. The binding of ^3H -flunitrazepam is displaced (more than 50%) by diazepam, RO 15-1788 and beta-carboline methyl ester. However, diazepam is 10-20 fold more potent than RO 15-1788 or beta-carboline methyl ester (IC_{50} 20 nM). The low affinity of beta-carboline methyl ester for the benzodiazepine binding sites in adrenal medulla was confirmed by direct binding measurement using ^3H -beta-carboline methyl ester. ^3H -RO 4864, the ligand for the peripheral benzodiazepine binding sites, failed to bind specifically to the adrenal medulla membranes in the concentration range from 0.4 to 200 nM. Moreover benzodiazepine receptors in adrenal medulla membranes can be photolabeled by ^3H -flunitrazepam while benzodiazepine receptors from kidney and liver are not. Hence ^3H -flunitrazepam cannot be considered to bind to a typical "peripheral" recognition site. Moreover this binding was increased by 30-40% by the addition of 100 μM GABA to the incubation medium. This increase was similar to that observed in brain. ^3H -muscimol also binds with high affinity to bovine adrenal medulla membranes and to membranes obtained from cultured chromaffin cells. The K_d is approximately 2nM and the B_{max} around 20 fmol/mg protein. Similarly to the GABA recognition sites of brain the binding of muscimol to medullary membranes was increased following treatment with 0.05% Triton-X 100. We are presently studying the effect of diazepam and muscimol on spontaneous and histamine and ACh-evoked release of catecholamines or enkephalin-like peptides from primary culture of adrenal chromaffin cells. Diazepam and muscimol (up to 10^{-6}M concentration) failed to alter the spontaneous and ACh-induced catecholamine release. However, preliminary experiments suggest that diazepam and muscimol in association change the spontaneous release of enkephalin-like peptides.

The present observations provide the first demonstration for a benzodiazepine GABA receptor complex of central type outside of the CNS. Moreover this study unequivocally provides evidence that this receptor complex does not include beta-carboline binding sites.

The strategic location of the benzodiazepine/GABA receptor complex on cell membranes which contain and release catecholamine- and enkephalin-like peptides may be of great importance in modulating the central behavioral effect of anxiolytic benzodiazepines.

Publications:

Kataoka, Y., Gutman, Y., Costa, E. and Guidotti, A. Benzodiazepine and muscimol binding sites in adrenal medulla: Receptors or drug acceptor sites? Neuroscience Abs., in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01572-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Endogenous effector for benzodiazepines		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) M.G. Corda Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <div style="display: flex; justify-content: space-between;"> <div> <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews </div> <div> <input type="checkbox"/> (b) Human tissues </div> <div> <input checked="" type="checkbox"/> (c) Neither </div> </div>		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> The presence in synaptic membranes of high affinity recognition sites for benzodiazepines which are capable of eliciting biochemical, physiological or behavioral responses when acted upon by specific ligands prompted the search for the identity of an endogenous ligand that operates in physiological conditions. The existence of an <u>endogenous modulator</u> for the <u>benzodiazepine-GABA receptor complex</u> was suggested by an early observation that the K_D for 3H-diazepam binding to GABA-free crude synaptic membranes was reduced by approximately 50% by repeated washes of the membranes with small concentrations of detergents. It was soon discovered that the membrane extract contained a peptide which produced a dose-related increase of K_D of diazepam binding without affecting the B_{max}. Recently we have developed a relatively simple procedure to isolate and purify to homogeneity this peptide. The peptide was termed <u>DBI (diazepam binding inhibitor)</u> and its extraction was routinely carried out using as starting material rat brain homogenized in hot (80°) 1N acetic acid. The purification was achieved using Sephadex G-100 and G-75 chromatography and reverse phase <u>HPLC</u>. </p>		

Project Description:

This project was done in collaboration with D. Konkel, Chemist; A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

Brain DBI (diazepam binding inhibitor) was purified to homogeneity as indicated by the presence of a single band of protein on SDS and on acidic urea gel electrophoresis and on three different columns and solvent systems for HPLC. It contains 104 amino acids with an abundance of lysine residues, is basic in nature, and has a MW of approximately 11,000 daltons. In addition, the HPLC peak with DBI activity contains tyrosine as a single carboxy terminus.

Experiments carried out to establish the amino acid sequence of this purified peptide revealed that the N-terminal amino acid is blocked. This block could not be resolved by several attempts carried out until now. The presence of 2-methionine in the molecule, allowed for the generation of 3 fragments following cyanogen bromide treatment. The sequence of the carboxy terminal fragment was determined, and a partial sequence of the middle fragment was determined. These sequences do not resemble any known mammalian peptide sequence. The fragment containing tyrosine as its carboxyterminus probably represents the terminal fragment of the molecule.

An important characteristic of DBI is that it is present in high concentrations (10-25 μ M in rat brain while only traces ($< 1 \mu$ M) were found in peripheral organs (liver, kidney, spleen). DBI appears to interact with benzodiazepine binding sites, competitively inhibiting the specific binding of 3 H-benzodiazepine and 3 H-beta-carboline derivatives. Because DBI inhibited the binding of beta-carbolines more effectively than that of benzodiazepines, an obvious question was, does DBI interact with benzodiazepine receptor acting like beta-carbolines (anxiogenic agents)? To answer this important question we have used binding and behavioral tests that are predictive for anxiolytic and anxiogenic effects.

In the first group of binding studies, we tested whether GABA shifts the DBI action on the benzodiazepine-GABA receptor complex. To this end, the binding of 3 H-RO 15-1788 was studied in presence or absence of 10^{-4} M GABA. 3 H-RO 15-1788 binding to rat brain synaptic membranes was insensitive to the presence or absence of GABA in the incubation medium. However, the displacement of 3 H-RO 15-1788 by diazepam was potentiated by GABA while that of beta-carboline ethyl ester was not. In this test DBI functions as a beta-carboline in that it displaces 3 H-RO 15-1788 in a GABA independent manner.

In another binding experiment we tested whether DBI alters 3 H-GABA binding. Although DBI in itself does not change the binding of 3 H-GABA, it blocks the benzodiazepine-induced increase of this binding. Again, DBI has an effect similar to that of the beta-carboline ethyl ester. Finally, the most direct approach was to test DBI in behavioral animal models that predict anxiolytic and anxiogenic activity.

When DBI is injected ICV into thirsty rats subjected to the Vogel test, it has a clear proconflict effect which is blocked by RO 15-1788. In addition, DBI fails to have any anticonflict action and actually blocks the anticonflict action of diazepam. The action of DBI is indistinguishable from that of the beta-carboline derivatives.

We intend to develop antibody against DBI and to study brain distribution of drug-induced alteration of DBI. The relatively large size and low potency of DBI in inhibiting

benzodiazepine and beta-carboline binding suggest that DBI could be the precursor of the real effector of the benzodiazepine recognition site.

The fact that DBI behaves like a beta-carboline derivative raises the possibility that it may not be the perfect endogenous ligand of benzodiazepine recognition site, for it may mimic beta-carboline derivatives rather than benzodiazepines. If this is the only endogenous ligand for the benzodiazepine recognition site present in rat brain then we must say that brain possesses only an endogenous anxiety mechanism and that it is an anxiogenic rather than anxiolytic mechanism. However the possibility that there are two sets of endogenous peptides, one mimicking the benzodiazepines and the other mimicking the beta-carbolines, cannot be excluded at this time.

Benzodiazepines are widely used to treat patients with pathological anxiety. Now a whole body of new research suggests that benzodiazepines correct an imbalance in the GABA benzodiazepine receptor system. This imbalance may be linked to naturally-occurring chemicals that work through the same brain cell mechanisms as benzodiazepines. Our work has uncovered what appears to be a natural substance that induces anxiety. If this observation is upheld, we believe the purification of such substance would revolutionize the treatment of anxiety.

Publications:

Guidotti, A., Forchetti, M.C., Corda, M.G., Konkell, D., Bennett, C.D., and Costa, E.: Isolation, characterization, purification to homogeneity of an endogenous polypeptide with agonistic action on benzodiazepine receptors. Proc. Natl. Acad. Sci. USA 80: 3531-3535, 1983.

Guidotti, A., Forchetti, M.C., Ebstein, B., and Costa, E.: Purification and characterization of an endogenous peptide putative effector for the benzodiazepine recognition site. In Usdin, E., Skolnick, P., Tallman, J.F., Greenblatt, D., and Paul, S.M. (Eds.): Pharmacology of Benzodiazepines. New York, MacMillan Press, 1983, pp. 529-535.

Guidotti, A., Corda, M.G., Vaccarino, F.M., and Wise, B.C.: Role of GABA-modulin and of an endogenous effector of beta-carboline binding sites in the GABA-benzodiazepine receptor interaction. In Bowery, N. (Ed.): Action and Interactions of GABA and Benzodiazepine. New York, Raven Press, 1983, in press.

Costa, E., Corda, M.G., and Guidotti, A.: On a brain polypeptide functioning as a putative effector for the recognition sites of benzodiazepine and beta carboline derivatives. Neuropharmacology, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01573-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) In vivo neurotransmitter receptor binding: Model for emission computed tomography		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) P. Ferrero Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) G. Di Chiro, NINCDS, Neuroradiology and Emission Computed Tomography Section, Bethesda, Md. LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 1.0	PROFESSIONAL: 1.0	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) High specific activity labeled ligands for neurotransmitter receptors can be used to characterize in vivo the status of the receptor. With an ideal ligand one can study regional distribution, saturability and pharmacological specificity of the binding and characterize the various functional states of the receptor. To study <u>DA receptors in vivo</u> , we have selected spiroperidol. This drug is an almost ideal probe for binding studies in vivo because it is poorly metabolized in brain and binds tightly to the DA receptors. The data obtained in animal models could be extended to measure DA receptor function in man injecting an appropriately labelled spiroperidol that could be used advantageously for <u>ECT scanning</u> .		

Project Description:

This project was done in collaboration with A. Guidotti, Chief of the Section on Neuroendocrinology and E. Costa, Chief of the Laboratory of Preclinical Pharmacology, SMRP, NIMH.

The content of authentic ^3H -spiroperidol and of its metabolites was measured in brain regions of rat, guinea pig and mouse receiving tracer doses of ^3H -spiroperidol intravenously (0.2 to 0.5 $\mu\text{g/kg}$). The time course of the ^3H -spiroperidol content of various brain regions shows that a steady state was maintained between 2 and 6 hrs; the lowest ^3H -spiroperidol content was attained in cerebellum where the value approached that of blood plasma. Since the cerebellum contains an insignificant number of dopamine receptors but many serotonin receptors and other sites that bind ^3H -spiroperidol, the ^3H -spiroperidol contained in cerebellum was considered background binding. In the striatum and olfactory tubercle of rats receiving two daily doses for 3 weeks of haloperidol or amphetamine the amount of ^3H -spiroperidol that binds *in vivo* is decreased or increased, respectively. If the kinetic characteristics of *in vivo* binding of ^3H -spiroperidol observed in the rat, guinea pig and mouse can be replicated in man using spiroperidol containing a γ - or α positron-emitting label, one might have a probe to study dopamine receptors *in vivo* with emission computed tomography scanning.

In vitro measurements of affinity constants for the binding of dopamine (DA) ligands to crude synaptic membranes are very useful in characterizing DA receptor recognition sites and in describing their density in various brain areas. For instance, when occupancy of DA recognition sites is decreased because of a lesion of nigrostriatal dopaminergic neurons or following chronic treatment with neuroleptic drugs, the number of ^3H -neuroleptic binding sites is increased. Moreover, binding studies in post mortem material have shown that schizophrenia and Parkinson affect ligand binding to DA recognition sites. Since contrasting opinions exist on the validity of extrapolating post mortem binding studies to the *in vivo* situation, it is hoped that emission computed tomography (ECT) scanning, in its two modalities, that with γ -emitters (single photon emission tomography (SPECT)) and that with positron emitters (PET) may be used to locate and diagnose DA receptor abnormalities *in vivo* in man.

Before undertaking human studies, it is necessary to evaluate animal models and adapt them to detect the *in vivo* modulation of receptor number or affinity that may occur in various pathological conditions. These models may help us to define whether sub and supersensitivity of receptors occurs in human pathology.

If it could be demonstrated that the ^3H -spiroperidol kinetic found in the three species applies also to man, an appropriately labeled spiroperidol could be used advantageously for ECT scanning. Considering that the steady state is reached at 2 hrs, a long living radiolabel (^{18}F as opposed to ^{11}C) would be preferable for PET studies. ECT scanning measurement could be carried out in the same individual at different intervals in order to establish a base line, and the administration of subpharmacological amounts of neuroleptics could be used to obtain a displacement curve from which to study the relative abundance, and the kinetic characteristics of DA_1 and DA_2 receptors.

Using the experience acquired with spiroperidol, we plan to extend this study to other receptor ligands. In particular we have now initiated studies on the *in vivo* binding of ^3H -muscimol to the GABA recognition sites.

Publications:

Moroni, F., Forchetti, C.M., Krogsgaard-Larsen, P., and Guidotti, A.: Relative disposition of the GABA agonists THIP and muscimol in the brain of the rat. J. Pharm. Pharmacol. 24: 676-678, 1982.

Ferrero, P., Vaccarino, F., Guidotti, A., Costa, E., and Di Chiro, G.: In vivo modulation of brain dopamine recognition sites: A possible model for emission computed tomography studies. Neuropharmacology, 1983, in press.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT		PROJECT NUMBER ZO1 MH 01574-01 SMRP
PERIOD COVERED October 1, 1982 to September 30, 1983		
TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.) Peptide ligands for nicotinic receptors		
PRINCIPAL INVESTIGATOR (List other professional personnel on subsequent pages.) (Name, title, laboratory, and institute affiliation) K. Kageyama Guest Worker SMRP NIMH		
COOPERATING UNITS (if any) None		
LAB/BRANCH Laboratory of Preclinical Pharmacology		
SECTION Neuroendocrinology		
INSTITUTE AND LOCATION NIMH, ADAMHA, NIH, Saint Elizabeths Hospital, Washington, D.C. 20032		
TOTAL MANYEARS: 0.6	PROFESSIONAL: 0.6	OTHER: 0
CHECK APPROPRIATE BOX(ES) <input type="checkbox"/> (a) Human subjects <input type="checkbox"/> (b) Human tissues <input checked="" type="checkbox"/> (c) Neither <input type="checkbox"/> (a1) Minors <input type="checkbox"/> (a2) Interviews		
SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.) <p> A bungarotoxin polypeptide different from α- and β-bungarotoxin was purified from bungarus multicintus venom. This peptide termed P-4 bungarotoxin contains 121 amino acids and blocks competitively nicotinic receptor function of adrenal chromaffin cells. Since there are no good nicotinic receptor ligands available to study nicotinic receptors in synaptic ganglia and CNS, <u>P-4 bungarotoxin</u> may represent an useful tool for these studies. </p>		

Project Description:

This project was done in collaboration with A. Guidotti, Chief of the Section on Neuroendocrinology, SMRP, NIMH.

The use of α -bungarotoxin has been instrumental for the identification, isolation, purification and characterization of nicotinic receptors. The action of α -bungarotoxin is well documented in electric organ of certain fish and eels and in the skeletal neuromuscular junction of mammalian and nonmammalian species. However conflicting results exist on the ability of α -bungarotoxin to interact with nicotinic receptors in central nervous system and in peripheral ganglia neurons. This uncertainty has greatly limited our program in understanding nicotinic receptors in mammalian CNS at a time in which alteration of acetylcholine transmission has been associated with Alzheimer's syndrome. The aim of this project has been that of identifying a peptide that specifically interact with nicotinic receptors of sympathetic ganglia or CNS neurons using as starting material bungarotoxin multicinctus venom. We have used as model for these studies the primary cultures of bovine chromaffin cells because they possess a nicotinic receptor link to the secretion of catecholamines. Using this model it was discovered that crude α -bungarotoxin preparations (purified from the venom by exchange column chromatography) inhibit the ACh or nicotine. However the potency of different lots of α -bungarotoxin was not related to the α -bungarotoxin peptide content but to that of another peptide (termed P-4 bungarotoxin) present as an impurity in the α -bungarotoxin preparations. P-4 bungarotoxin was isolated and purified to homogeneity by HPLC. Homogeneity was established by a variety of means including polyacrylamide gel electrophoresis, HPLC and end carboxy group analysis. Purified P-4 contains approximately 121 amino acid residues and it is different in its amino acid composition and MW from α -bungarotoxin and β -bungarotoxin. End group analysis of P-4 bungarotoxin reveals that glycine is the carboxy terminus. Sixty of the 121 amino acids have been now sequenced.

P-4 bungarotoxin ($IC_{50} = 10^{-9}M$) competitively blocked the ACh induced release of endogenous catecholamines but failed to block the KCl-induced catecholamine release. Although P-4 bungarotoxin is endowed with phospholipase A-2 activity, its effect on ACh-evoked catecholamine release persists also when the phospholipase activity is blocked (99.9%) by treatment of the toxin with p-bromophenacyl-bromide.

This research may represent the basis for the identification of an antagonist ligand of the nicotinic receptors in mammalian CNS. Since the function of nicotinic receptors in CNS has never been carefully investigated, the approach we have taken can be of great importance in understanding the role of this receptor in the control of brain function. If we will be successful in labelling this ligand we may have an interesting tool to study in detail the role of nicotinic receptor in Alzheimer disease.

We propose:

- 1) To develop a method to obtain high specific activity labeled P_4 bungarotoxin without lost of its biological activity.
- 2) Explore the behavioral and biochemical effect of P_4 bungarotoxin injected directly into the lateral ventricles in experimental animals.

Publications:

Kageyama, H., and Guidotti, A.: Effect of modulators of nicotinic receptor function on endogenous and radiolabelled catecholamine release from primary cultures of adrenal chromaffin cells. J. Neurosci. Methods, submitted, 1983.

Saiani, L., Kageyama, H., and Guidotti, A.: Purification and characterization of a bungarotoxin polypeptide which blocks nicotinic receptor function in primary culture of adrenal chromaffin cells. Mol. Pharmacol., submitted, 1983.

NIH Library, Building 10
National Institutes of Health
Bethesda, Md. 20205



<http://nihlibrary.nih.gov>

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

JRSI
1985

NIH LIBRARY



3 1496 00312 5948